The effect of nutritional support and GH/IGF-I treatment on glutamine metabolism in the critically ill.

Jackson, Nicola Clare

THE EFFECT OF NUTRITIONAL SUPPORT AND GH/IGF-I TREATMENT ON GLUTAMINE METABOLISM IN THE CRITICALLY ILL.

NICOLA CLARE JACKSON

Department of Diabetes, Endocrinology and Internal Medicine

Guys, Kings and St Thomas' School of Medicine

Kings College London

A thesis submitted in part fulfilment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF LONDON

April 2000

ABSTRACT

Previous studies have suggested that glutamine may be a conditionally essential amino acid during critical illness. The aim of this study was to use L-[2-^{15}N] glutamine as a tracer to investigate glutamine kinetics in critically ill patients and to determine whether nutritional support and combined growth hormone/insulin-like growth factor-I (GH/IGF-I) treatments have any adverse or beneficial effects on glutamine metabolism. Initial studies in healthy controls indicated glutamine appearance rate (Ra_{gln}) expressed per kg body weight was lower in elderly (>60 years) compared to young (<35 years) subjects ($p<0.05$), but Ra_{gln} expressed per kg lean body mass was not different. Glutamine kinetics were measured in critically ill patients and matched healthy controls. Although Ra_{gln} was similar in both groups, the proportion of Ra_{gln} arising from protein breakdown (B_{gln}; $p<0.05$) and the glutamine metabolic clearance rate (MCR_{gln}; $p<0.001$) were higher, whereas plasma glutamine concentration was lower in the patients than in the control group ($p<0.001$). The lower plasma glutamine concentration and unaltered plasma Ra_{gln} suggest that although B_{gln} was higher this was insufficient to meet the increased demand for glutamine in these patients.

The effects of total parenteral nutrition (TPN), and TPN supplemented with glutamine (TPNGLN, 0.4 g $kg^{-1}day^{-1}$), on glutamine metabolism were studied in critically ill patients. Ra_{gln} and MCR_{gln} were not altered by either treatment. Plasma glutamine concentration ($p<0.001$) and glutamine uptake (Rd_{gln}; $p<0.05$) both increased with glutamine supplementation. These results suggest there is an increased requirement for glutamine in critically ill patients.

The effects of treatment with TPNGLN and combined GH (0.2 IU $kg^{-1}day^{-1}$)/IGF-I (160 μg $kg^{-1}day^{-1}$) therapy (TPNGLN+GH/IGF-I) were also investigated. TPNGLN+GH/IGF-I did not alter Ra_{gln} and the increase in glutamine concentration ($p<0.05$) and Rd_{gln} ($p<0.05$) were similar to that achieved in the TPNGLN group. This suggests that combined GH/IGF-I treatment did not have adverse effects on glutamine metabolism when given with glutamine supplemented TPN.

INDEX **Page**

List of Figures

List of Tables **Page**

ABBREVIATIONS USED IN THIS THESIS

AAA	aortic abdominal aneurysm
ACTH	adrenocorticotrophic hormone
ADP	adenosine diphosphate
ALS	acid-labile subunit
ANOVA	analysis of variance
APE	atom percent excess
APACHE II	acute physiology and chronic health evaluation
ARDS	acute respiratory distress syndrome
ATP	adenosine triphosphate
B_{gln}	glutamine release from protein breakdown
B_{leu}	leucine release from protein breakdown
BCAA	branched chain amino acids
BMI	body mass index
C_{gln}	glutamine catabolism
C_{leu}	leucine catabolism
CI	colour index
CRH	corticotrophin releasing hormone
CRP	C-reactive protein
CV	coefficient of variation
D_{gln}	de novo glutamine synthesis
DNA	deoxyribonucleic acid
EI	electron impact
Endo Ra_{gln}	endogenous glutamine appearance rate
F	isotope infusion rate
GCMS	gas chromatography mass spectrometry
GC-C-IRMS	gas chromatography-combustion-isotope ratio mass spectrometry
GI	gastrointestinal
GH	growth hormone
GHBP	growth hormone binding protein
GHRH	growth hormone releasing hormone
[gln]	glutamine concentration
I_{gln}	dietary glutamine intake

I_{leu}	dietary leucine intake
ICU	intensive care unit
IGF-I	insulin-like growth factor I
IGFBP	insulin-like growth factor binding protein
k	molar ratio of glutamine to leucine content of body protein
KIC	α-ketoisocaproic acid
LBM	lean body mass
MCR_{gln}	glutamine metabolic clearance rate
MTBSTFA	N-methyl-N-(*tert*-butyl dimethylsilyl)-trifluoroacetamide
m/z	mass to charge ratio
Q_{gln}	whole body glutamine turnover
Q_{leu}	whole body leucine turnover
QC	quality control
Ra	appearance rate
Ra_{gln}	glutamine appearance rate
Rd_{gln}	glutamine disappearance rate
Rb	peak area ratio of baseline sample
Rs	peak area ratio of enriched sample
RNA	ribonucleic acid
S_{gln}	incorporation of glutamine into protein
S_{leu}	incorporation of leucine into protein
SE	standard error of mean
SIM	selected ion mointoring
rT_3	reverse tri-iodothyroxine
T_3	tri-iodothyroxine
T_4	thyroxine
TBDMS	*t*-butlydimethylsilyl
TIC	total ion chromatograph
TISS	therapeutic intervention scoring system
TPN	total parenteral nutrition
TPNGLN	total parenteral nutrition supplemented with glutamine
TSH	thyroid stimulating hormone
-ve	negative
+ve	positive

Acknowledgements

I would like to thank the following people:-

- my supervisor, Dr Margot Umpleby, for her constant encouragement and invaluable advice
- Dr Paul Carroll for his involvement in the clinical studies
- Dr David Russell-Jones for his continued enthusiasm and support
- Dr Fariba Shojaee-Moradie and Mr Abu Fofanah for their help with the hormone assays
- the nurses and staff in the Intensive Care Unit for their patience
- the healthy controls, especially my father and the rest of the elderly group for volunteering for the tracer studies
- my friends in the Department of Endocrinology, Diabetes and Internal Medicine for their encouragement, advice and for making working in the department so enjoyable

Finally, I would like to thank Dr David Treacher (Clinical Director of the Intensive Care Unit) and Professor Peter Sönksen (Head of the Department of Endocrinology, Diabetes and Internal Medicine) for their support without which this research project would not have been possible.

Chapter 1

INTRODUCTION

Glutamine

Glutamine is a five carbon amino acid with an anhydrous molecular weight of 146.15 (Figure 1.1). At physiological pH the carboxyl group of glutamine carries a negative charge whereas the amino group is protonated, this results in the molecule carrying a net zero charge and the classification of glutamine as a neutral amino acid. Because of its two nitrogen side chains, an α amino group and an amide group, glutamine can also be classified as an amino acid amide.

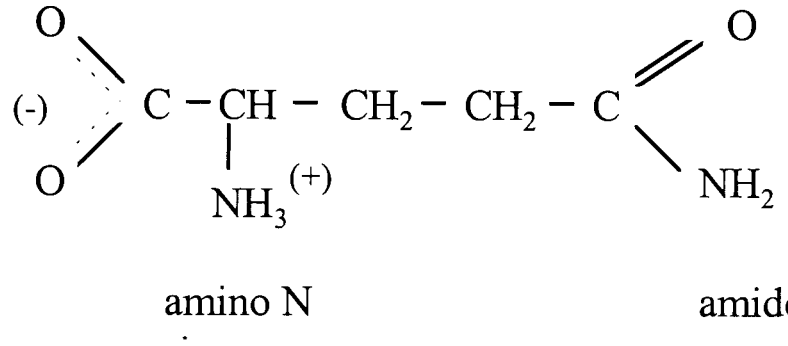


Figure 1.1. The molecular structure of glutamine.

Glutamine is the most abundant free amino acid in the human body. In plasma glutamine has the highest amino acid concentration (typically 400-900 μmol l^{-1}) and accounts for approximately 20 % of the total circulating amino acid pool (Smith and Wilmore 1990). In skeletal muscle, which contains about half the total free amino acids in the body, glutamine constitutes approximately 60 % of the free amino acid pool, excluding taurine (Bërgstrom et al 1974).

As glutamine can be synthesised endogenously and has such a high natural abundance, it has been traditionally classified as a non-essential amino acid. This definition implies sufficient glutamine can be produced endogenously to meet the glutamine requirements of the body. However, free glutamine concentrations are very labile and marked decreases have been reported in a variety of catabolic states (Vinnars et al 1975, Askanazi et al 1980). This suggests that during serious illness a deficiency in glutamine availability may develop and has led to the idea that glutamine is a

conditionally essential amino acid (Lacey and Wilmore 1990). In the last two decades glutamine metabolism, its influence on metabolic control and the body's response to dietary supplementation has become a major experimental and clinical research area.

Glutamine metabolism

The importance of glutamine was emphasised by Hans Krebs in 1980 when he stated, "Most amino acids have multiple functions but glutamine appears to be the most versatile".

Glutamine is an important intermediate in a number of metabolic pathways and plays a key role in nitrogen homeostasis. Some of the major functions of glutamine metabolism in man are listed below:-

- Because of its two amino moieties glutamine is considered to be the principal carrier of nitrogen from peripheral tissues to the visceral organs.
- Glutamine plays an important role in hepatic ureagenesis.
- Glutamine plays a key role in acid-base homeostasis as an important substrate for renal ammoniagenesis.
- Glutamine is an important energy source for rapidly growing cells such as the mucosal cells of the intestinal mucosa, lymphocytes and most cultured cells.
- Glutamine is also a nitrogen donor in the synthesis of nucleotides such as ATP, and purine and pyrimidine synthesis and is therefore essential for cell proliferation.
- Glutamine is an important precursor for the antioxidant glutathione.

There are numerous enzymes involved in glutamine metabolism; the two key enzymes which are involved in the inter-conversion of glutamine and glutamate, are shown in Figure 1.2. Phosphate-dependent glutaminase is bound to the inner mitochondrial membrane and catalyses the hydrolysis of glutamine to glutamate and ammonia. There appear to be two major forms of glutaminase, the renal and the hepatic forms which have different kinetic properties and protein structure (Neu et al 1996). Glutamine synthetase is located primarily in the cytoplasm and catalyses the biosynthesis of glutamine from glutamate and ammonia. This reaction is energy dependent, requiring one mole of ATP per mole of glutamine synthesised. The different intracellular location of these two enzymes is appropriate for their metabolic functions; glutaminase

catalyses the utilisation of glutamine as an energy source whereas glutamine synthetase produces glutamine for cytosystolic protein and nucleotide synthesis (Neu et al 1996).

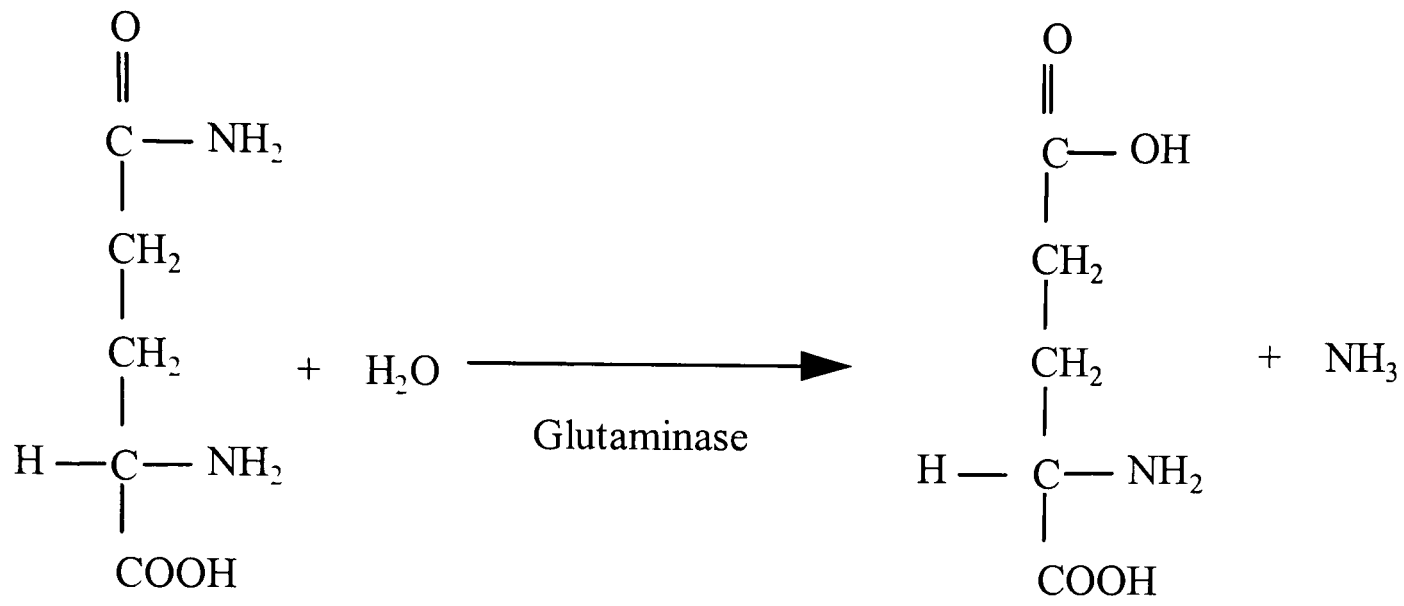


Figure 1.2. Reactions catalysed by glutaminase and glutamine synthetase.

Although most tissues contain both enzymes many are either predominantly glutamine consumers and contain relatively high amounts of glutaminase (i.e. gut mucosal cells, endothelial cells, lymphocytes, renal tubular cells) or net glutamine producers and contain relatively high amounts of glutamine synthetase (i.e. skeletal muscle, lung, brain and heart). Because of the large mass of skeletal muscle in the body, skeletal

muscle glutamine synthetase catalyses the synthesis of almost 30 % of the total circulating glutamine (Neu et al 1996). The liver can act as either a net glutamine consumer or producer depending on the metabolic state, and regulates the activity of glutaminase or glutamine synthetase accordingly.

Glutamine and muscle

Skeletal muscle is the largest protein pool in the body and accounts for 30-40 % of the total body weight in humans. Assuming the free glutamine concentration in all skeletal muscle is about 20 mmol per litre of intracellular water, it has been estimated that skeletal muscle (25 kg of muscle, ~80 % water, in a 70 kg man) contains about 400 mmoles or 60 g of free glutamine (Souba 1992). This glutamine pool remains fairly constant during conditions of health but marked falls have been observed in catabolic conditions associated with protein wasting (Vinnars et al 1975, Askanazi et al 1980, and Roth et al 1982).

Amino acids are not released from muscle in the same proportion in which they are present in the contractile proteins. In the post-absorptive state more than 50 % of the amino acids released from skeletal muscle consist of glutamine and alanine (Ruderman and Berger 1974). This is due to the de novo synthesis of these two amino acids from amino acids and other substrates. The branched chain amino acids and glutamate are the most important nitrogen donors for the de novo synthesis of glutamine (Darmaun and Dechelotte 1991).

Animal studies have suggested a relationship between glutamine concentration and protein synthesis. Infusion of glutamine reduced the post-operative efflux of amino acids from the dog hind limb (Kapadia et al 1985). A positive correlation has been shown between free glutamine levels in muscle and the protein synthesis rate in the perfused rat hind limb in the presence, or absence, of insulin (MacLennan et al 1987). Using a similar rat skeletal muscle model Jepson and co-workers (Jepson et al 1988) demonstrated the relationship between muscle glutamine concentration and protein synthesis rate existed in a number of catabolic states including dietary restriction or endotoxin injection. This correlation is specific for glutamine and has not been observed with other amino acids. The fall in muscle glutamine concentration was highly correlated with the decrease in ribosomal concentration and activity (Jepson et

al 1988). MacLennan and co-workers have also demonstrated glutamine inhibition of protein degradation, again in the rat skeletal tissue model. Elevated concentrations of glutamine in the perfusate diminished the dilution of the [^{15}N] phenylalanine tracer, suggesting an inhibition of muscle protein breakdown (MacLennan et al 1988).

However, several groups have failed to observe a correlation between muscle glutamine concentration and the protein fractional synthetic rate in various models of stress in the rat. Infusion of glutamine in post-absorptive rats in vivo did not stimulate protein synthesis (Garlick and Grant 1988). In a more recent study a glutamine synthetase inhibitor (methionone sulphoximine) was used to decrease glutamine levels in rats (Olde Damink et al 1999). The decrease in plasma glutamine (40 %) and intracellular muscle glutamine (60 %) levels had no effect on whole body protein turnover or muscle protein kinetics (measured using [2,6-^{3}H] phenylalanine). This study suggests that it is unlikely that the intracellular muscle glutamine concentration is a major regulating factor in muscle protein kinetics

Glutamine and the intestinal tract

Early in vitro experiments investigating the oxidation of various ^{14}C labelled substrates by incubated ileum discs showed that glutamine was oxidised to an equal or greater extent than glucose in a number of animal species, including the monkey, rat and rabbit (Neptune 1965). Windmueller and Spaeth demonstrated the importance of glutamine as a major respiratory fuel for the gut in vascularly perfused gut preparations from fasted rats (Windmueller and Spaeth 1974) and in situ, autoperfused, isolated jejunum sections in both fasted and fed rats (Windmueller and Spaeth 1978 and 1980). Most of the glutamine uptake occurs in the small intestinal mucosal cells which have high glutaminase activity (Windmueller and Spaeth 1974).

In the post-absorptive state the gut lumen is relatively empty so the vast majority of glutamine must be extracted from the blood stream. In the post-prandial state glutamine is also taken up by the gut from the lumen, across the brush border. The fate of glutamine carbons has been traced by infusing [U-^{14}C] glutamine into the lumen of rat isolated jejunum sections and measuring the specific activity of carbon products released into the venous blood (Windmueller and Spaeth 1975). Approximately one third (34 %) of the infused glutamine was recovered unchanged in the venous blood

collected from the segments. About 56 % of the carbon from the metabolised glutamine was recovered in the blood as CO_2, 20 % as organic acids (mostly lactate) and 15 % in other amino acids (proline, citrulline, alanine, ornithine and glutamate). A similar distribution of products was found in earlier experiments when the [U-^{14}C] glutamine was administered to the vascular side of the rat intestine (Windmueller and Spaeth 1974). These results suggest glutamine is metabolised similarly whether it enters the mucosal cells from the luminal side or the blood stream. Nitrogen taken up by the jejunum as glutamine can be accounted for by the net release of ammonia, citrulline, alanine, proline, glutamate and ornithine (Windmueller and Spaeth 1980).

Most of the information on glutamine metabolism by the intestinal tract has been derived from in vitro and in vivo animal experiments. The relative inaccessibility of the portal vein has made the in vivo study of intestinal glutamine metabolism in humans difficult. Early balance studies in humans, which sampled blood from the brachial artery and an hepatic vein, demonstrated net uptake of glutamine by the splanchic bed as a unit, but did not separate the contributions of the gut and liver (Marliss et al 1971, Felig et al 1973). Felig and co-workers also took blood samples from the brachial artery and the hepatic portal vein in a small group (n=4) of patients undergoing elective cholecystectomy. The positive arterio-portal venous glutamine balance (533 ± 30 vs. 460 ± 24 μmol l^{-1}) indicated net glutamine uptake by intestinal tissues (Felig et al 1973). However sampling from the portal vein includes other portal drained viscera such as the spleen and pancreas.

Several more recent studies have confirmed the prominent role of the small intestine in glutamine metabolism in humans. Using a whole body stable isotope tracer technique Darmaun and co-workers (1991a) showed that whole body glutamine utilization was reduced by 20 % in patients who had undergone extensive small bowel resection. In contrast the whole body glutamine appearance rate (Ra_{gln}) was increased (13 %) in adult patients with active coeliac disease (Messing et al 1998). Glutamine fractional extraction rates (i.e. percentage of glutamine extracted from arterial blood) have been measured in patients undergoing elective surgery for gastrointestinal malignancies (Van der Hulst et al 1997). Extraction rates of 24, 9, and 8 % were obtained for the jejunum, ileum and colon, respectively, suggesting glutamine is a more important fuel

in the proximal gut compared with the distal gut. Splanchnic bed utilisation of glutamine tracers, given by nasogastric tubes to fasted adults, has been estimated at 54 % (Matthews et al 1993) and 73 % (Hankard et al 1995).

An important function of the gut is to prevent the translocation of bacteria and endotoxins from the gut lumen into the systemic circulation that can trigger sepsis (Meakins and Marshall 1986). The mucosal epithelium is a dynamic rapidly dividing tissue which is highly dependent on adequate nutritional supply. Delivery of adequate amounts of glutamine may be necessary for maintenance of the gut mucosal integrity. Intestinal glutamine uptake may be concentration dependent, Van der Hulst and co-workers showed glutamine extraction by the ileum was correlated with arterial glutamine concentrations (Van der Hulst et al 1997). A reduced uptake of glutamine by the gut may be present in disease states characterised by low plasma glutamine concentrations. This may be particularly important during catabolic illnesses when there may be severe glutamine depletion and oral nutrition may be interrupted. Standard parenteral nutrition, which does not contain glutamine, has been associated with marked atrophy of the intestinal mucosa. The addition of glutamine to parenteral nutrition prevents the deterioration of gut permeability and preserves mucosal structure (Van der Hulst et al 1993).

Glutamine and the liver

The liver plays a central role in glutamine metabolism, as it can act as an organ of net glutamine uptake, release, or balance depending on several factors such as ammonia concentration, pH and the metabolic requirements. There are two distinct hepatocyte populations which contain different enzyme systems.

The portal blood flow initially comes into contact with the periportal cells (~ 93 % of hepatocytes) which contain glutaminase and the enzymes of the urea cycle. These low affinity, high capacity cells take up glutamine and ammonia and synthesise urea. The glutaminase in the periportal hepatocytes is structurally, kinetically and immunologically different to glutaminase found in other tissues, such as the kidney. Unlike renal glutaminase hepatic glutaminase is activated by ammonia and is not inhibited by glutamate. Periportal glutamine breakdown increases flux through the urea cycle by amplifying the mitochondrial supply of ammonia for the first enzyme of

the urea cycle, carbamoylphosphate synthetase. In the perivenous cells (~7 % of hepatocytes) close to the hepatic vein glutamine synthetase synthesises glutamine from ammonia and glutamate. These high affinity cells act as "scavengers" for ammonia that has escaped the initial extraction for urea synthesis in the periportal cells.

Under normal non-stressed physiologic conditions (balanced acid-base situation) the breakdown and synthesis of glutamine by the liver (intercellular glutamine recycling) are roughly matched and the liver acts as an organ of glutamine balance (Häussinger 1990). Flux through the intercellular glutamine cycle increases in situations known to increase urea production, such as an increase in the portal ammonia load. However, during acidosis urea synthesis is decreased and glutamine synthesis is favoured in order to provide glutamine for the renal ammoniagenesis.

Glutamine and the kidneys

In the renal tubular cells glutamine deamidation (by the enzyme glutaminase) generates ammonia which diffuses into the lumen of the tubules where it combines with a proton to form the ammonium ion. The ammonium ion is excreted in urine along with an anion such as chloride. During metabolic acidosis the rate of renal glutamine consumption is increased to support ammoniagenesis and the kidneys become the major organ of glutamine utilisation (Souba 1992). In extreme acidic conditions glutamate can be deaminated (via the enzyme glutamate dehydrogenase) to generate additional ammonia (Lacey and Wilmore 1990). In contrast, renal glutamine uptake is decreased during alkalosis.

Glutamine and the immune system

Glutamine is a key substrate for both lymphocytes and macrophages. In vitro experiments have shown glutamine to be essential for the normal functioning of these cells in the immune response (Parry-Billings et al 1990). Glutamine plays a dual role providing an energy source via oxidation and nitrogen precursors for the synthesis of purine and pyrimidine nucleotides for DNA and RNA.

Lymphocytes that have not been exposed to an immune stimulus are often called "resting" lymphocytes which implies that they exist in a relatively quiescent metabolic state. The metabolism of these cells is accelerated in response to an immune stimulus,

to support cell division and the secretion of antibodies, cytokines and other soluble protein mediators. Macrophages are terminally differentiated white cells which do not characteristically divide in response to an immune stimulus. However, when "active" they have high rates of RNA and protein synthesis, associated with production of secretory proteins (interleukins and lysosomal enzymes) and membrane recycling (phagocytosis).

In lymphocytes glutamine is only partly oxidised, yielding mainly glutamate, aspartate and lactate, a process termed "glutaminolysis" (Ardawi and Newsholme 1985). Very little glutamine is oxidised via acetyl-CoA and the "classic" Krebs cycle, despite the presence of the Krebs cycle enzymes. The possible reason for this is that generation of a high ATP concentration would inhibit key reactions in glutaminolysis (e.g. oxoglutarate dehydrogenase). In vitro experiments have shown the rate of glutaminolysis and hence glutamine utilisation is much higher than is necessary for the provision of intermediates for biosynthetic pathways (Szondy and Newsholme 1989). It has been suggested that the high flux of glutamine allows optimal response when the immune system is activated, so that high rates of purine and pyrimidine synthesis can be achieved without depleting the concentrations of the metabolic precursors (glutamine and aspartate).

Glutamine transport

Amino acids are transported into and out of cells by distinct transport proteins located in the cell plasma membrane. Table 1.1 lists some of the major amino acid transport systems for glutamine and other neutral amino acids (McGivan 1996, Palacín et al 1998). None of the transport systems are specific for glutamine or any amino acid, but they transport structurally related amino acids. Many of these transporters are sodium dependent, and require initial binding of the sodium ion prior to binding of the amino acid. The electrochemical potential gradient of sodium ions drives the amino acid accumulation across the plasma membrane. The identification of amino acid transporters was originally based on functional characteristics such as their kinetically determined amino acid specificity, ion dependence and pH dependence (Ahmed et al 1993). Since the beginning of the 1990s complementary DNA clones have been isolated for several members of the systems L and ASC transporter families, and the first member of the system A family has recently been identified (Varoqui et al 2000).

A number of different transport systems with overlapping specificity may occur in the same cell membrane. The three major amino acid transport systems for neutral amino acids in mammalian tissues are systems A, ASC and L, which are present in almost all cell types (McGivan and Pastor-Anglada 1994). Some of the amino acid transporters appear to be more tissue specific. For example in hepatocytes glutamine uptake is mediated by system N in both rats (Kilberg et al 1980) and humans (Bode et al 1995). A system with similar properties has been identified in rat skeletal muscle and has been designated system N^m (Hundal et al 1987). System N^m has also been characterised in sarcolemmal vesicles isolated from human skeletal muscle, however in humans system N^m is pH sensitive (Ahmed et al 1993).

An important feature of system A is that its activity is highly regulated in many cell types (McGivan and Pastor-Anglada 1994, Palacín et al 1998). Insulin, glucagon, catecholamines and glucocorticoids have been shown to upregulate system A activity (Palacín et al 1998). System N activity also appears to be highly regulated. In hepatocytes system N is upregulated by hormones such as insulin, glucagon and glucocorticoids (Bode et al 1990). Growth hormone treatment has been shown to decrease system A activity in both human and rat hepatocytes, but does not affect system N (Pacitti et al 1992). In muscle cells cultured in glutamine free media system N^m shows increased transport activity to compensate for the reduced glutamine supply (i.e. adaptive up regulation), but systems A, ASC and L are not affected (Tadros et al 1993).

Table 1.1. Some of the major transport systems for neutral amino acids (Modified from McGivan 1996, Palacín et al 1998)

System	*Specificity*	*Distribution*	*Comments*
Na^+ dependent			
System A	Small aliphatic amino acids Preferred substrates: alanine, serine, glutamine	Widespread	pH sensitive. Highly regulated, inducible by hormones, amino acid deprivation, osmotic stress.
System ASC	Small aliphatic amino acids Preferred substrates: alanine, serine, cysteine	Widespread	pH insensitive. Not usually inducible.
System N	Glutamine, histidine, asparagine	Liver	pH sensitive. Inducible by hormones, osmotic stress.
System N^m	Glutamine, histidine, asparagine	Skeletal muscle	pH sensitive (human), insensitive (rat). Inducible by insulin amino acid deprivation, osmotic stress.
System B	Most neutral amino acids	Renal and intestinal brush borders	Transepithelial transport.
Na^+ independent			
System L	Mainly branched chain and aromatic amino acids	Widespread	Not usually inducible.

Critically ill patients

Critical illness is defined as any condition requiring support of failing vital organs, without which death would occur (Van den Berghe 1999). This definition includes a multitude of catabolic states such as sepsis, trauma, burns and major surgery (Jenkins and Ross 1996). The role of the intensive care unit (ICU) is to manage patients with acute life threatening illnesses and to restore their previous quality of health and life (Griffiths et al 1995). Admission of patients to an ICU is usually unplanned and typically features a period of crisis involving shock or sepsis followed by a period of organ support to maintain life while normal function is re-established (Griffiths et al 1995). This support can involve the use of mechanical aids, for example mechanical ventilators or dialysis machines, or pharmacological agents, such as inotropes or vasopressors (Van den Berghe 1999). Critically ill patients are often elderly, not physically active, and suffer from an underlying disease, with a generally compromised health state. These patients are distinct from metabolically healthy patients with stable circulation and minimal preoperative blood loss undergoing planned operative intervention (standardised surgical procedures: e.g. elective cholecystectomy).

Metabolism and endocrine changes in critical illness

Critical illness is associated with profound hormonal and metabolic alterations which result in the development of a hypercatabolic state. Increased amounts of free amino acids are mobilised from skeletal muscle for high priority use in other tissues such as the gastrointestinal tract, liver, and kidneys (Kinney and Elwyn 1983). The amino acids are used as metabolic fuels, substrates for gluconeogenesis, ammoniagenesis and anabolic processes such as synthesis of acute phase proteins and wound healing. The clinical observation of muscle wasting in ICU patients is well documented and is of considerable clinical importance as skeletal muscle fatigue results in difficulties in weaning the patient off a ventilator (Gamrin et al 1996). The progressive loss of muscle mass is associated with disturbed wound healing and tissue repair and an increased susceptibility to infection

In the short term the changes observed in the neuroendocrine axis of critically ill patients are generally adaptive and provide optimal intravascular volume, perfusion pressure and substrate availability to facilitate healing and recovery (Rolih and Ober 1995). However if the illness is prolonged these changes can contribute to a worsened

clinical outcome. The complete spectrum of changes seen in critically ill cannot be explained by the neuroendocrine response alone (Pittoni et al 1997). In addition to hormones the cytokines and the inflammatory mediators (prostaglandins, leukotreines and platelet activating factor) mediate the hypercatabolism seen in severe illness. The exact relationship between the cytokines, the inflammatory mediators and the traditional hormones in the generation and maintenance of the hypercatabolism of critical illness has not yet been established.

It is very difficult to study hormonal changes in critically ill patients for a number of reasons:-

- the heterogeneity of the patient groups.
- lack of knowledge of the pre-illness metabolic state which may include underlying malnutrition.
- the use of therapeutic agents which can potentially affect neuroendocrine function such as dopamine (Van den Berghe et al 1994a and 1994b).
- there may be damage to organs directly involved in the endocrine response or to those involved in hormone metabolism.

A number of mechanisms may be involved in the endocrine changes seen in critically ill patients. These include changes in hormone production rates, half-lives, binding protein levels and affinities, receptor binding, post-receptor signalling mechanisms and feedback loops, some of these changes are summarised in Table 1.2 (Jenkins and Ross 1996).

Table 1.2. Characteristics of hormone changes seen in critical illness (Jenkins and Ross 1996).

The pituitary-adrenal axis
• Cortisol level increased with loss of diurnal variation
• Biphasic ACTH response initially high then falls
• Cortisol binding globulin level decreased
• Glucocorticoid receptor affinity decreased
The thyroid axis
• Free T_3 decreased
• Free T_4 decreased or normal
• rT_3 increased
• TSH levels decreased or normal
• Thyroxine binding globulin affinity decreased
• Thyroid hormone receptor expression increased
The growth hormone-insulin-like growth factor-I axis
• GH levels increased
• IGF-I levels low
• IGFBP-1 levels high and IGFBP-3 levels low
• IGFBP-3 protease present

The pituitary-adrenal axis

The major glucocorticoid in humans is cortisol. In healthy adults cortisol production depends on stimulation of the adrenal cortex by adrenocorticotrophic hormone (ACTH) also known as corticotrophin. ACTH is secreted by the corticotrophs of the anterior pituitary in a distinctive diurnal pattern which cortisol secretion mirrors. The corticotrophs are stimulated by corticotrophin releasing hormone (CRH), from the hypothalamus, to produce ACTH. Glucocorticoids provide negative feedback on ACTH and CRH secretion and in addition CRH secretion is modulated by stress. The majority of circulating cortisol (80-90 %) is bound to serum proteins, primarily corticosteroid binding globulin and albumin and as such is not active (Rolih and Ober 1995).

Numerous studies have reported elevated cortisol levels in critically ill patients and shown that the degree of hypercortisolemia is related to the severity of illness (Jurney et al 1987, Vermes et al 1995). There are several reasons for the hypercortisolaemia including an increased production rate, altered pituitary glucocorticoid feedback and loss of the circadian rhythm (Ross et al 1991b, Reincke et al 1993). In the initial phase of critical illness the high serum cortisol concentrations are associated with increased levels of ACTH. However, after 3-5 days the ACTH levels fall to below normal levels, whereas the cortisol levels remain elevated (Vermes et al 1995). Cortisol protein binding levels are also low in critical illness, so there may be a greater elevation in free cortisol levels than indicated by the increase in total cortisol levels (Perrot et al 1993).

Hypercortisolism increases gluconeogenesis and lipolysis and decreases protein synthesis to provide metabolic substrates for vital organs and postpone anabolism in other tissues (Van den Berghe et al 1998). In addition the immune response is inhibited by cortisol which is interpreted as an attempt to protect against over-responsiveness of the inflammatory cascade (Munck et al 1984).

The thyroid axis

Thyroxine (T_4) is the major circulating thyroid hormone (normal range 5-10 $\mu g\ dl^{-1}$), however more than 99 % circulates bound to binding proteins (globulin and albumin) and is physiologically inactive. In the peripheral tissues T_4 is converted to the more active form tri-iodothyroxine (T_3). The majority of circulating T_3 originates from this

peripheral conversion and less than 20 % is directly secreted by the thyroid gland. Normal total T_3 levels are 100-200 ng dl^{-1} and similarly more than 99 % is protein bound. T_4 can also be converted to reverse T_3 (rT_3) which only has 1 % of the activity of T_4. The production of thyroid hormones is stimulated by thyroid stimulating hormone (TSH) from the anterior pituitary. The thyroid hormones have direct effects on metabolism by stimulating intracellular metabolic activity and indirect effects by potentiating the effects of other hormones such as the catecholamines and insulin.

The changes in the hypothalamo-pituitary-thyroid axis in critical illness are known as non-thyroidal illness or "sick euthyroid syndrome" (Rolih and Ober 1995). The most common thyroid abnormality associated with critical illness is characterised by low T_3, normal T_4 levels, increased rT_3 and normal or low TSH levels. In about 20 % of ICU patients the T_4 levels are also low. The initial changes in thyroid function during critical illness appear to be mainly changes in peripheral metabolism, binding and receptor occupancy of thyroid hormones (Van den Berghe et al 1998). Although TSH levels usually remain normal, T_3 levels drop rapidly, partly due to decreased conversion of T_4 to T_3 (Chopra et al 1985) and/or increased turnover of thyroid hormones (Kaptein et al 1981). The magnitude of the fall in T_3 levels within the first 24 hours appears to reflect the severity of the illness (Rothwell and Lawler 1995). The increase in rT_3 levels is partly due to decreased degradation (Chopra et al 1985).

The changes in thyroid hormone levels seen in critically ill patients have been suggested to be an attempt to reduce energy expenditure and alleviate catabolism (Jenkins and Ross 1996).

The growth hormone-insulin-like growth factor-I axis

Growth hormone (GH) is a 191 amino acid single chain peptide secreted by the somatotropes of the anterior pituitary gland. The GH profiles of normal adults consist of peaks every 3-4 hours alternating with virtually undetectable levels between the peaks, with the majority of the GH secretion being at night. This pulsatile GH secretion is controlled by the stimulatory action of hypothalamic growth hormone releasing hormone (GHRH), the inhibitory action of somatostatin and several neural and hormonal feedback loops which act directly, or indirectly on pituitary GH synthesis and/or release. A significant proportion (45-80 %) of the GH in plasma is attached to

binding proteins (GHBP) (Baumann et al 1988). Bound GH is cleared more slowly than free GH and prolongs GH bioavailability by creating a circulating reservoir of GH (Baumann et al 1987). High affinity GHBP represents the extracellular domain of the GH receptor and is thought to reflect GH receptor expression or abundance (Baumann 1999).

Although for many years GH was only considered to be important in the growth of children it is now well recognised that GH has important metabolic actions in adults, particularly in the maintenance of normal body composition. GH deficient adults have reduced lean body mass, increased fat mass (Salomon et al 1989) and decreased bone mineral density (Holmes et al 1994).

In critically ill patients GH profiles are characterised by reduced pulse amplitude and elevated baseline levels and are associated with low circulating levels of insulin-like growth factor I (IGF-I) (Ross et al 1991a). The elevated GH levels and low IGF-I levels are interpreted as resistance to GH. Reduced GH binding protein activity, which is thought to reflect decreased GH receptor expression, has also been reported in critically ill patients (Ross et al 1991b).

In recent years, with the development of recombinant DNA technology, large quantities of synthetic GH have become available for therapeutic use. GH has been demonstrated to have protein anabolic effects in normal subjects (Fryburg et al 1991) and GH deficient adults (Russell-Jones et al 1993). Administration of GH has been shown to improve nitrogen balance in healthy adults receiving hypocaloric intravenous nutrition (Manson and Wilmore 1986) and in patients receiving hypocaloric or full intravenous support (Ziegler et al 1988, Douglas et al 1990, Ponting et al 1990, Berman et al 1999). GH therapy has also been shown to improve nitrogen balance in ICU patients receiving nutritional support (Ziegler et al 1990b, Jeevanandam et al 1996).

Many of the actions of GH are mediated by IGF-I expression in tissues or circulating IGF-I produced by the liver. The bioavailability and clearance of IGF-I are regulated by a series of binding proteins (IGFBP-1 to 6), the most important of which appear to be IGFBP-1 and 3.

The vast majority of circulating IGF-I is associated with IGFBP-3 which further associates with a glycopeptide called the acid-labile subunit (ALS). The synthesis of these three polypeptides is normally increased by GH. Ternary complexed IGF-I is estimated to have a half-life of 15 hours, whereas binary IGF-IGFBP-3 complexes are estimated to have half-lives of 15-25 minutes and free IGF-I has a half-life of only a few minutes. In acute critical illness the concentration of IGFBP-3 is low due to an increase in a specific protease (Timmins et al 1996). This protease may explain the shorter half-life reported for subcutaneous IGF-I injections in post-operative patients compared to normal subjects (Miell et al 1992). IGFBP-1 is found at higher levels in conditions of stress and is inversely modulated by insulin (Ross et al 1991b).

Recombinant human IGF-I was also available for use in human studies, but compared to GH there have been relatively few clinical trials with IGF-I since its commercial development was stopped in 1995. A 16 hour IGF-I infusion in calorically restricted subjects improved nitrogen balance (Clemmons et al 1992). Infusion of IGF-I overnight in fasted, normal subjects demonstrated that like insulin IGF-I inhibits protein degradation and protein synthesis (Turkalj et al 1992). However, if sufficient substrate is provided in the form of an amino acid infusion, IGF-I infusion stimulates protein synthesis but has no effect on protein degradation in normal post-absorptive adults (Russell-Jones et al 1994).

Glutamine metabolism in critical illness

The metabolic response to critical illness is characterised by net catabolism of protein which results in an increased flow of amino acids from skeletal muscle, mainly glutamine and alanine, to the visceral organs. It has been suggested that muscle protein degradation provides glutamine for rapidly dividing cells such as immunocytes and enterocytes and possibly cells involved in wound healing (Soeters 1994). In addition, glutamine utilisation by the kidney is increased as the ammonia produced facilitates the excretion of acid equivalents (Van Acker et al 1997)

As early as 1975 Vinnars et al reported that muscle free glutamine concentrations were significantly reduced (by more than 35 %) in patients 3 days after major abdominal surgery compared to healthy subjects, whilst plasma glutamine levels remained unchanged. In healthy individuals undergoing moderate elective surgery this decrease

in muscle free glutamine levels was detectable 12 hours postoperatively (Essen et al 1992). The decrease in muscle free glutamine was shown to be still present on the 10th day following surgery and levels were slowly restored to preoperative levels over 20 to 30 days (Petersson et al 1992).

Critically ill patients have an extremely low level of free glutamine in skeletal muscle. Gamrin et al 1996 measured free glutamine concentrations in muscle biopsy and blood samples from a group of critically ill patients who had been in an ICU for 3 to 14 days. Intracellular glutamine concentrations were depleted by 72 %, whereas plasma levels were only decreased by 23 %. The decrease in muscle free glutamine concentration is greater than for any other amino acid and persists during recovery when other amino acids have normalised. Although critically ill patients are a very heterogeneous group the decrease in muscle glutamine concentration is quite uniform between patients and is independent of the severity of illness (Gamrin et al 1996).

The fall in free glutamine concentration suggests endogenous glutamine synthesis is unable to meet the increased metabolic demand. Inability to meet the increased glutamine demand may have severe consequences for tissues consuming glutamine at high rates such as the gut and the immune system (Van Acker et al 1997). This has led to questioning of the non-essential character of glutamine (i.e. glutamine is "conditionally essential") and further research into the possible benefits of an exogenous supply of glutamine during times of increased metabolic demand.

ICU and nutritional support

Nutritional support is an important therapy which has greatly improved the care of critically ill patients. Nutritional and metabolic support is provided during critical illness to reduce the net negative nitrogen and energy balance (Evans 1994). A variety of parenteral and enteral solutions containing various amounts of amino acids, glucose, lipid, micronutrients and electrolytes have been used (Souba 1997). Parenteral nutrition is used when enteral nutrition is not suitable or as a supplement to enteral nutrition if adequate nutrient administration is not possible by enteral nutrition alone (Cerra et al 1997). Although parenteral solutions are not as nutritionally complete as enteral formulas, nutritional targets are more often achieved by the use of total parenteral nutrition (TPN). However TPN is associated with increased incidence of

infection and is known to result in atrophy of intestinal mucosa and to promote bacterial translocation from the intestinal lumen (Neu et al 1996).

Despite aggressive enteral and parenteral nutritional support, it is often difficult to attenuate the catabolic response in critically ill patients (Streat et al 1987). Strategies to prevent the loss of lean tissue are constantly under investigation and include the provision of "conditionally essential amino acids" such as glutamine, and the use of anabolic hormones, such as GH and IGF-I.

Glutamine supplements

In early parenteral nutrition solutions, in which the amino acids were derived from casein hydrolysates, glutamine was present at low concentrations reflecting its levels in most animal and plant proteins (~7 % of total amino acids) (Lacey and Wilmore 1990). When synthetic amino acids preparations beacame available glutamine was omitted from TPN solutions for two main reasons:-

- Firstly, glutamine administration was not considered necessary because glutamine was originally classified as a non-essential or nutritionally dispensable amino acid. This classification implied glutamine could be synthesised in adequate quantities from other amino acids and precursors.

- Secondly, there were a number of pharmaceutical reasons for not including glutamine in commercially available TPN. Free glutamine is considered to be unstable in aqueous solution at room temperature and is also heat labile, undergoing ring condensation to form pyroglutamate and ammonia. In addition the low solubility of free glutamine (36 g l^{-1} at 20 °C) considerably increased the volume of fluid to be infused into the patients (Fürst et al 1989).

Much research has been directed to the use of more chemically stable sources of glutamine such as dipeptides and analogues of glutamine as supplements for TPN. Dipetides such as L-alanyl-L-glutamine and glycyl-L-glutamine are stable when heat sterilised, and are well tolerated and are rapidly converted to glutamine when administered intravenously to heathy adults (Albers et al 1988, Fürst et al 1989, Hübl et al 1989). These dipetides also have the advantage of increased solubility for

example the solubility of L-alanyl-L-glutamine is 568 g l^{-1} in H_2O at 20 °C (Fürst et al 1989). Ornithine-α-ketoglutarate and α-ketoglutarate have also been used as precursors of glutamine to supplement TPN (Wernerman et al 1989, 1990). α-Ketoglutarate can be transaminated readily to glutamate which can then be amidated by glutaminase synthetase.

However, Hardy and co-workers have recently shown that the widely reported that instability of free glutamine during storage in aqueous solutions is a misconception, and glutamine is stable if the correct pharmaceutical and sterilisation techniques are used (Hardy et al 1993). Aseptically prepared and filter-sterilised glutamine solutions can be stored in oxygen impermeable bags for up to 30 days at 4 °C (Hardy et al 1992). Glutamine losses were less than 0.05 % per day, pyroglutamate formation was less than 0.02 % per day and ammonia formation less than 0.01 mmol l^{-1} day^{-1}. Stability of the glutamine was not adversely affected by incorporation into TPN; the glutamine content remained at 98.2 % of the original level after 30 days.

Concern also existed about glutamine administration because of its close biochemical relationship to ammonia. Ziegler and colleagues investigated the clinical safety, pharmacokinetics and metabolic effects of L-glutamine administration in healthy adults (Ziegler et al 1990a). These studies showed administration of glutamine both orally and intravenously as a short-term infusion (4 hours) was well tolerated without clinical or biochemical side effects. The safety of glutamine as a component of parenteral nutrition (5 days) in normal subjects and patients undergoing bone marrow transplantation (3-5 weeks) has also been confirmed (Ziegler et al 1990a). The glutamine solutions were cold sterilised by membrane filtration and stored up to 10 days at 4 °C. Solution stability studies demonstrated no appreciable ammonia generation during storage.

In vivo measurement of glutamine kinetics

Blood and tissue concentrations of metabolites are carefully controlled by various regulatory processes and many vary little in healthy adults. Observations based on these apparently static metabolite levels provide no insight into the rates of endogenous production or disappearance via metabolism. Much of the information on glutamine metabolism has been obtained from in vitro and in vivo animal experiments. In vivo

measurement of human glutamine metabolism has relied on two important techniques: 1) organ balance techniques and 2) whole body tracer dilution methods.

Organ balance techniques

Using the organ balance technique the net balance (net uptake or release) of glutamine across a specific organ is calculated from the arterio-venous substrate concentration differences and blood flow rates. The first published data on human glutamine metabolism used this technique and showed glutamine to be released from muscle and taken up by the splanchnic bed and kidney (Marliss et al 1971, Felig et al 1973, and Owen and Robinson 1963). Major drawbacks of this technique are that it requires difficult catheterisation which has limited its use in humans. It also measures the net effects of glutamine handling by the organ, and does not provide information on the rates of glutamine uptake or release.

Whole body tracer dilution methods

Quantitative information about the turnover of a metabolite in vivo can be obtained by labelling one or more of its constituent atoms with an isotope of that atom to form a tracer. The tracer can be injected or infused intravenously, or ingested orally, and its metabolism can be followed by monitoring the levels in blood, urine or breath samples at timed intervals (Umpleby and Sönksen 1987). The tracer and the non-labelled metabolite (tracee) are assumed to be indistinguishable to biological systems.

The whole body appearance rate (Ra) of an individual amino acid can be determined in vivo by measuring the systemic dilution of a continuously infused tracer. In this basic model the assumption is made that the Ra of the amino acid and the infusion of the tracer occur into, and sampling is from, a single, homogeneous, instantly mixing pool (Wolfe 1992). The tracer is infused at a constant rate over a period of several hours until an isotopic equilibrium or a "steady state" level is achieved in plasma. Measurement of the tracer enrichment in plasma samples yields a rising curve which approaches a plateau at steady state. During this plateau the rate of appearance of the tracee and tracer into the plasma pool are equal to their rate of removal from the system, so the isotopic enrichment remains "steady". Only small numbers of samples need to be taken during the isotopic plateau. The infusion time can be shortened by the injection of a priming dose of tracer immediately before the beginning of the infusion.

The tracer is usually administered in an antecubital vein and blood samples are taken from a contra-lateral peripheral vein. Subjects are normally studied following an overnight fast to ensure that the measurements are made in a steady state (Umpleby and Sönksen 1987). It is generally assumed that there is no recycling of the tracer during the time course of the study. A drawback of this method is that it provides little information of contribution of specific organs to the whole body changes.

The use of stable isotopes as kinetic tracers in vivo predates the use of radioisotope tracers by almost 20 years (Wolfe 1992). However, because of the limited supplies of stable isotope tracers at the time and their relatively difficult methods of analysis, most kinetic studies used radioisotope labelled tracers. Increasing awareness of the health hazards of radioisotopes severely limited the total dose that could be administered, thus restricting studies to short periods and precluding repeat studies. Furthermore radioisotope tracers cannot be used in children or women of childbearing age. Radioisotope tracer studies of glutamine metabolism were difficult to interpret. ^{14}C was considered an inappropriate label, because glutamine, glutamate and α-ketoglutarate all have the same five carbon skeleton and the metabolism of these three compounds could not be confidently separated (Golden et al 1982).

In the past two decades stable isotopes have been increasingly used as tracers in metabolic research studies in man. Advances in electronic instrumentation and computer technology led to the development of simple to operate, relatively inexpensive, mass spectrometers for the measurement of isotopic enrichment in biological samples. Also stable isotope tracer supplies have generally become cheaper and more widely available.

The majority of elements exist in a stable form, the term stable isotope is used to describe those that occur at low natural abundance e.g. ^{13}C 1.1 %. These can be used as tracers when incorporated into metabolite molecules at high enrichments. The use of stable isotopes overcomes the ethical and safety problems of radioisotope tracers and has several other advantages. These advantages include the ability to measure isotope enrichment and substrate concentration in the same sample, the ability to label the metabolite with multiple tracers and the ability to infuse several labelled metabolites simultaneously in the same subject. In addition the half-life of

^{13}N (9.9 minutes) and ^{15}O (2.07 minutes), the radioisotope equivalents to ^{15}N or ^{17}O and ^{18}O, were considered to be too short to be used in metabolic studies of several hours duration (Young and Ajami 1999). However these radioisotopes are now being employed in studies using positron emission tomography. A minor disadvantage is that stable isotopes are naturally occurring so tracer enrichments must be measured above this basal or background level. The infusion of a stable isotope tracer cannot normally be considered to be massless and the calcuated Ra has to be corrected for the tracer infusion.

Mass spectrometry

When stable isotope tracers are used in metabolic studies the steady state enrichment of the metabolite is most often determined by a mass spectrometer. There are many types of mass spectrometers that have widely varying capabilities and applications but they all measure the abundance of ionised particles at specified masses. Because of the versatility and small sample size requirements (μl of plasma) the isotopic enrichment in plasma samples is usually measured by combined gas chromatography mass spectrometry (GCMS) with a quadrupole mass analyser. The GC inlet allows separation of the metabolite of interest from other metabolites in a complex biological sample. In the MS the molecules are ionised which allows them to be controlled by voltages and electrical fields. The MS is generally operated in the selected ion monitoring (SIM) mode in which the analyser acquires data from a few specific masses, increasing the accuracy. GCMS analysis requires the formation of volatile derivatives of the metabolite. The derivatising agents often contain isotopes which also contribute to the background ratio for example the popular *tert*-butyldimethylsilyl derivatives contain Si and C which both have relatively high proportions of stable isotopes (^{13}C 1.1 %, ^{29}Si 4.7 %, ^{30}Si 3.1 %).

AIMS

The aims of the present study were:-

- To establish a protocol using L-[2-^{15}N] glutamine as a tracer to measure whole body glutamine turnover.

- To measure whole body glutamine turnover in critically ill patients in the ICU at St Thomas' Hospital.

- To investigate the effects of glutamine supplementation on glutamine metabolism in critically ill patients receiving parentetal nutrition.

- To investigate the additional effects of GH and IGF-I treatment on glutamine metabolism critically ill patients receiving TPN supplemented with glutamine.

Chapter 2

CLINICAL METHODOLOGY

Subjects and ethical consent

Normal subjects (n=12) were recruited from staff in the Department of Diabetes, Endocrinology and Internal Medicine and their relatives. Critically ill subjects were recruited from newly admitted patients in the ICU (Mead Ward) at St Thomas' Hospital in whom the clinical decision had been made to use TPN. Exclusion criteria included diabetes, malignancy, renal failure, myopathy, and liver disease. Nineteen severely ill patients were initially studied, two patients died before the completion of the second study, data is only presented from the seventeen patients who survived to complete both studies. However as indicated in Table 7.1, one patient died later in the ICU.

All normal subjects provided informed written consent and informed written consent was obtained from relatives of the ICU patients. The studies were approved by the West Lambeth Health Authority Ethics Committee.

The metabolic tracer studies in the healthy subjects and patients required the assistance of a clinically qualified colleague, Paul Carroll. The protocol included the use of L-[1-^{13}C] leucine as a tracer to permit quantitation of whole body protein turnover. The protein turnover data will form part of Paul Carroll's MD thesis and therefore is not discussed in the present thesis. However, the leucine appearance rate (a measure of whole body protein breakdown) has been used to estimate the proportion of the glutamine appearance rate (Ra_{gln}) derived from protein breakdown and de novo synthesis (Chapter 3).

Preparation of tracer solutions

L-[2-^{15}N] glutamine (99 %) and L-[1-^{13}C] leucine (99 %) were purchased from MassTrace (Somerville, MA, USA). Sterile solutions of the tracers were prepared by the Pharmacy Department at St Thomas' Hospital. Accurately weighed amounts of glutamine or leucine were dissolved in known amounts of sterile, pyrogen free 0.9 % saline to give 25 mg ml^{-1} and 15 mg ml^{-1} solutions respectively. These solutions were filtered (0.22 μm) into sterile vials which were then aseptically sealed; a random

sample of vials underwent sterility and pyrogen testing. The glutamine vials were stored at -20 °C and the leucine vials at room temperature.

On the day of the study the stock tracer solutions were diluted with 0.9 % saline in 50 ml sterile syringes to give infusion rates of 2.5 mg kg^{-1} hr^{-1} for glutamine and 1 mg kg^{-1} hr^{-1} for leucine.

Study Protocol

Normal subjects

The normal subjects were admitted to the research area of the Diabetes and Endocrine Day Centre following an overnight fast. Height and weight were recorded and body composition was measured by bioelectrical impedance (Tanita, Tokyo, Japan) (Luskaski et al 1986). Subjects were studied in a semi-recumbent position. A cannula was inserted into an antecubital vein for the isotope infusion and another cannula was placed in a superficial vein of the contra-lateral hand for blood sampling. "Aterialised" venous blood was obtained by placing the hand in a heated box (air temperature 60 °C) during the sampling period (Abumrad et al 1981). Following baseline sampling a 4 hour continuous infusion of [2-^{15}N] glutamine 2.5 mg kg^{-1} hr^{-1} and a primed (1 mg kg^{-1}) constant infusion of [1-^{13}C] leucine 1 mg kg^{-1} hr^{-1} were started using a Harvard pump (Harvard Apparatus, MA, USA). Blood samples were taken at 210, 215, 220, 225, 230 and 240 minutes for steady state measurement of glutamine and α-ketoisocaproic acid (KIC) enrichment and glutamine concentration.

Blood samples were also taken at baseline and steady state for the measurement of substrate and hormone levels (glucose, insulin, C-peptide, GH, IGF-I, IGFBP-1 and 3, cortisol, glucagon, thyroid hormones, amino acids).

To establish the optimal infusion protocol, extra blood samples were taken at 30, 60, 90, 120, 150, 180, 250, 260, 270, 280, 310 and 330 minutes for the measurement of glutamine enrichment in five of the normal subjects.

Critically ill patients

All the patients were fasted for at least 12 hours before the start of the first study (Study 1). An identical infusion protocol to the normal subjects was used in the critically ill patients however, the blood samples were taken from in-dwelling arterial lines. At the end of the baseline study the nutritional ± hormonal treatment was commenced. Following 72 hours of treatment a second turnover study (Study 2) was performed using the same tracers and infusion rates as the initial study. In this second study the patients were not fasted as nutritional support was continued throughout the protocol (Figure 2.1). The patients were sedated and mechanically ventilated (Servo 900; Siemens, Berlin, Germany) throughout both studies.

Sample collection and storage

Blood samples were taken with sterile syringes and added to vacutainers containing various anticoagulants. Blood for glutamine, KIC and amino acid samples were collected in lithium heparin tubes. Glucose samples were collected in fluoride oxalate tubes and C-peptide samples in ethylenediaminetetra-acetate tubes with added Trasylol. Plasma samples were mixed and kept on ice prior to centrifugation at 1500 g, 4 °C for 10 minutes. Blood samples for the measurement of insulin, cortisol, thyroid hormones, GH, IGF-I, IGFBP-1 and 3 were all collected in plain tubes. Serum samples were left at room temperature for 30 minutes prior to centrifugation at 1500 g, 4 °C for 10 minutes. Plasma and serum samples were transferred to small plastic tubes for storage.

For glutamine analysis, two separate 0.5 ml plasma aliquots were mixed with 100 µl internal standard (100 nmol L-[U-$^{13}C_5$] glutamine, Bioquote Ltd., UK) before storage. Plasma for amino acid analysis was deproteinised with an equal volume of 10 % sulphosalicylic acid containing the internal standard norleucine (100 nm ml^{-1}). The samples were incubated on ice for 30 minutes before re-centrifugation after which the supernatant was transferred to small plastic tubes for storage. All samples were stored at -70 °C until analysis.

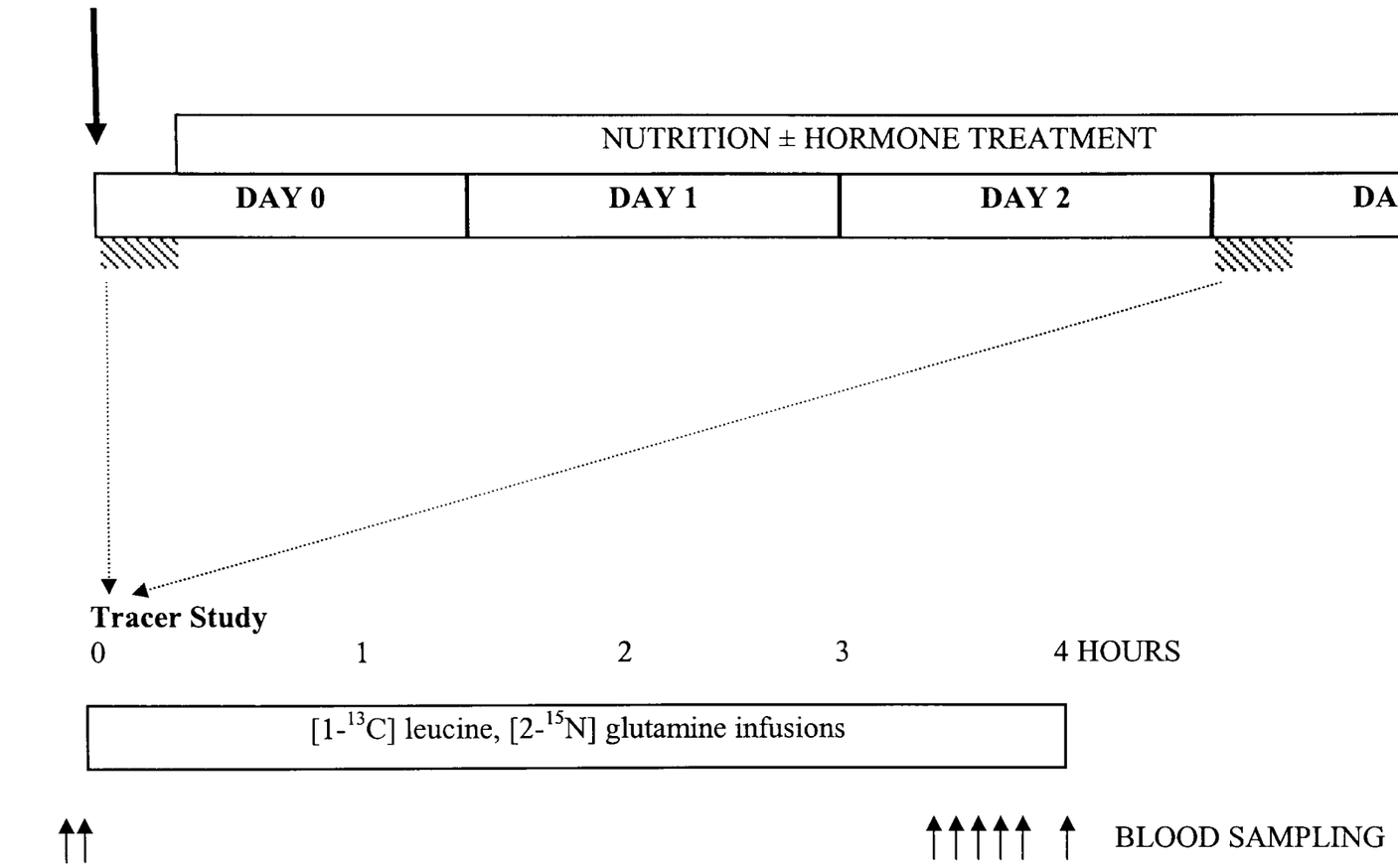


Figure 2.1. Study protocol for critically ill patients.

Measurement of lean body mass

The body composition of the healthy subjects was measured using the technique of bioelectrical impedance analysis (Tanita, Tokyo, Japan) (Luskaski et al 1986). This method depends on the principal that fat is a very poor conductor of electricity whereas the fat free mass or lean body mass (LBM) is a good conductor as it contains virtually all the water and conducting electrolytes in the body. Prediction equations which take into account body weight, height and gender, are used to convert the measured impedance into an estimate of total body water. The hydration of lean tissue is assumed to be constant allowing the calculation of LBM. Fat mass is then calculated as the difference between total body weight and LBM.

APACHE II and TISS scores

APACHE II (Acute Physiology and Chronic Health Evaluation) and TISS (Therapeutic Intervention Scoring System) are the systems for scoring the severity of illness in ICU patients which are currently used at St Thomas' Hospital.

The APACHE II score consists of a total of three scores (Knaus et al 1985):-

1) twelve routine physiological measurements
2) age
3) previous health status

The physiological measurements consist of variables such as core temperature, blood pressure, heart rate, respiratory rate and specific blood tests which include markers of the current health status (e.g. white blood cell count, arterial pH, serum sodium and potassium levels). The worst score for each of the physiological measurements (either increase or decrease from a standard reference range) over a 24 hour period is recorded. There are precise criteria for defining the presence of a chronic illness for example renal insufficiency is present if the patient is receiving long term haemodialysis or peritoneal dialysis. The chronic illness is also scored depending on whether the patient has undergone planned elective surgery or is a non-surgical or emergency surgical patient. The APACHE II score is used to predict outcome and define the ICU case mix.

The TISS score is based on a series of eighty statements which assess the level of therapeutic intervention required (Cullen et al 1974). For example these include the

use of central lines, the need for organ support such as dialysis, nutritional support (enteral or parenteral), and the level of nursing required (e.g. 1:1). The statements are scored a value of 1-4 depending on the level of managed input they require. The TISS score is used as it correlates well with ICU costs.

Chapter 3

ANALYTICAL METHODS

Mass spectrometry analysis

Measurement of glutamine enrichment and concentration

Background

In 1982 Golden and co-workers reported the first study of whole body glutamine kinetics in humans using a stable isotope tracer. The method involved the enzymatic hydrolysis of amide nitrogen of glutamine to ammonia, oxidation of the resulting ammonia to N_2 gas and measurement of ^{15}N enrichment by dual inlet, isotope ratio mass spectrometry. This procedure was long, tedious and the volume of blood required (20 ml per sample) made it impractical for clinical studies.

Several investigators have reported methods for measuring ^{15}N glutamine enrichments by GCMS (Yudkoff et al 1982, Nissim et al 1984, Darmaun et al 1985, Anderson et al 1987). GCMS analysis should allow amino acid separation and isotopic enrichment to be measured in a single step. However, GCMS requires the derivatisation of amino acids before analysis and unfortunately most derivatisation reactions deamidate glutamine to glutamate so plasma glutamine and glutamate need to be separated prior to derivatisation (Darmaun et al 1985).

In the present study the tertiary-butyldimethylsilyl derivatives (TBDMS) were chosen for quantification of and isotopic enrichment measurements of glutamine by GCMS (Chaves Das Neves and Vasconcelos 1987, Anderson et al 1987). TBDMS derivatives are popular in GCMS, the electron impact (EI) mass spectra are normally dominated by large $[M-57]^+$ ions produced by loss of a tertiary butyl group. These ions contain the entire skeleton of the original compound and are very suitable for quantitative measurements by selected ion monitoring when using stable isotope tracers (Schwenk et al 1984). The added advantage of this derivative is that it leaves the glutamine molecule intact and should allow the measurement of ^{15}N enrichment of glutamine by GCMS without the need for separation from glutamate prior to derivatisation. Glutamine concentration was determined by reverse isotope dilution using L-[U-$^{13}C_5$] glutamine (98 %, Bioquote Ltd., Yorks., UK) as the internal standard (Darmaun et al 1985, Anderson et al 1987).

Materials

All chemicals and reagents were purchased from Sigma-Aldrich Ltd. (Dorset, UK) or BDH-Merck Ltd. (Leics.,UK) unless stated otherwise. Helium (CP grade) and N_2 (oxygen free) gases were purchased from BOC Gases (Lancs., UK)

Instrumentation

All GCMS analysis was performed on a Hewlett Packard 5971A Mass Selective Detector (Hewlett-Packard, Berkshire, UK). The system was equipped with a 5890 Series II gas chromatograph fitted with a 25 m, 0.25 mm i.d., RtX-1 capillary column (crossbonded dimethyl polysiloxane; Thames Chromatography, Berks., UK) using helium as the carrier gas (8 psi, 1 ml min^{-1}). The transfer line to the mass spectrometer was held at 280 °C and the column was directly coupled to the ion source of the mass spectrometer. Samples (1µl) were injected by hand or a Hewlett Packard 7673 injector in the splitless mode. The injector temperature was held at 250 °C. The column was held at 100 °C for 0.75 minutes, then programmed at 25 °C min^{-1} to 300 °C and held for 4 minutes at 300 °C. The mass spectrometer was operated in the positive ion EI mode (70eV). The EI mass spectra were obtained by scanning at 1.0 scan s^{-1} from m/z 50 to 650.

Derivatisation of standards

Stock solutions (1 mg ml^{-1}) of glutamine, [2-^{15}N] glutamine, [U-$^{13}C_5$] glutamine and glutamate were prepared in distilled water, aliquoted and stored frozen at -70 °C. Working solutions in the range 10 - 100 µg ml^{-1} were prepared from these.

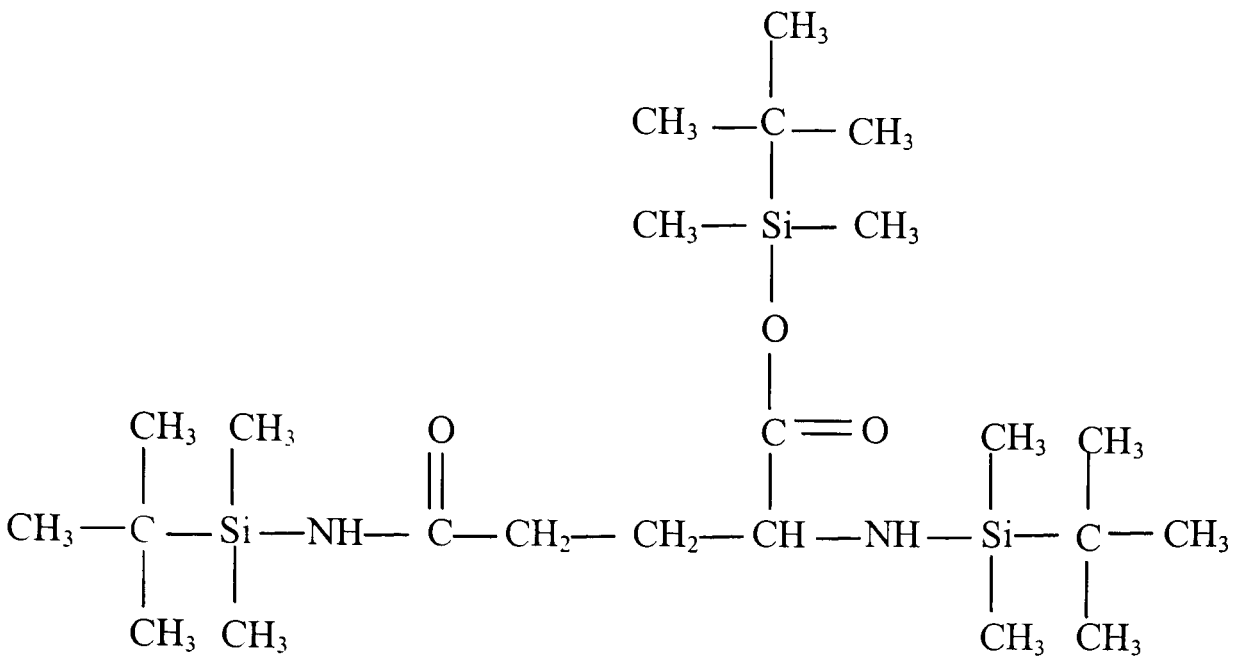
As a first stage in the validation of the glutamine assay, TBDMS derivatives of glutamine (and glutamate) standard solutions were prepared and the EI mass spectra recorded on the GCMS. This allowed GC conditions to be established that separated glutamine from glutamate and confirmation of the ions to monitor for quantification and isotopic enrichment measurements.

Aliquots of the glutamine and glutamate standards were derivatised with 100 µl N-methyl-N-(*tert*-butyldimethylsilyl)-trifluroacetamide (MTBSTFA) and

100 µl acetonitrile at 120 °C for 2 hours and scanned on the above GCMS system. The structure of the TBDMS derivative of glutamine is shown in Figure 3.1, the derivatised glutamine contains three TBDMS groups attached to the carboxyl, amino and amide groups.

The tri-TBDMS glutamine was chromatographically separated from the tri-TBDMS glutamate, the total ion chromatograms (TIC) showed a peak at 9.5 minutes for glutamate and 10.2 minutes for glutamine (Figure 3.2). There were no corresponding peaks in the derivatisation blanks (Figure 3.3). The electron impact mass spectra of tri-TBDMS glutamine and tri-TBDMS glutamate are shown in Figure 3.4 and Figure 3.5 respectively. The glutamine mass spectra showed a weak molecular ion peak at m/z 488 and a weak $[M-15]^+$ ion at m/z 473. The glutamate mass spectra also showed a molecular ion peak at m/z 489 and a weak $[M-15]^+$ ion at m/z 474. Both spectra showed the $[M-159]^+$ ions, corresponding to $[M-CO_2TBDMS]$, at m/z 329 for glutamine and m/z 330 for glutamate. Except for the low mass ion observed at m/z 73 the most abundant ion in each spectrum was the $[M- butyl]^+$ ion at m/z 431 for glutamine, and at m/z 432 for glutamate. Figure 3.6 and Figure 3.7 show the EI mass spectra of [2-^{15}N] glutamine and [U-$^{13}C_5$] glutamine. The 431 ion was shifted to (i) 432 in the spectrum of [2-^{15}N] glutamine and (ii) 436 in the spectrum of [U-$^{13}C_5$] glutamine.

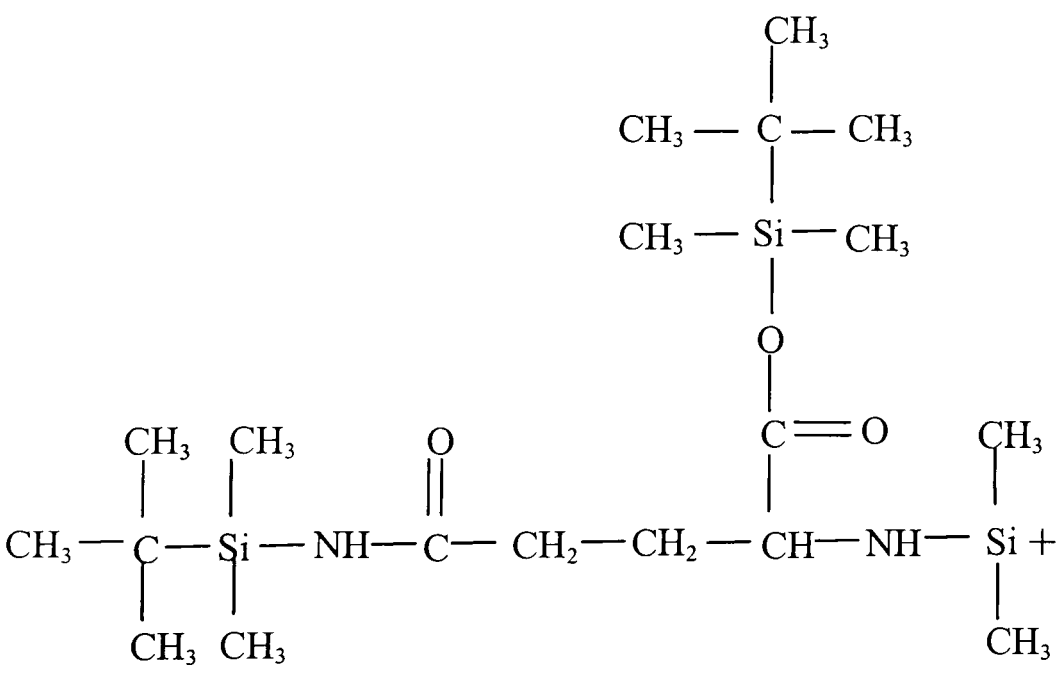
The $[M-butyl]^+$ fragment ion was chosen for subsequent selected ion monitoring for the quantification and isotopic enrichment measurements. Ions were monitored at m/z 431, 432, and 436 for glutamine, [2-^{15}N] glutamine and [U-$^{13}C_5$] glutamine respectively, using a dwell time of 20 msec giving 8.55 cycles per sec.



$C_{23}H_{51}N_2O_3Si_3$

molecular weight 488

$-[C_4H_9]$



$C_{19}H_{43}N_2O_3Si_3$

m/z 431

Figure 3.1. The tri-TBDMS derivative of glutamine and the $[M\text{-butyl}]^+$ ion.

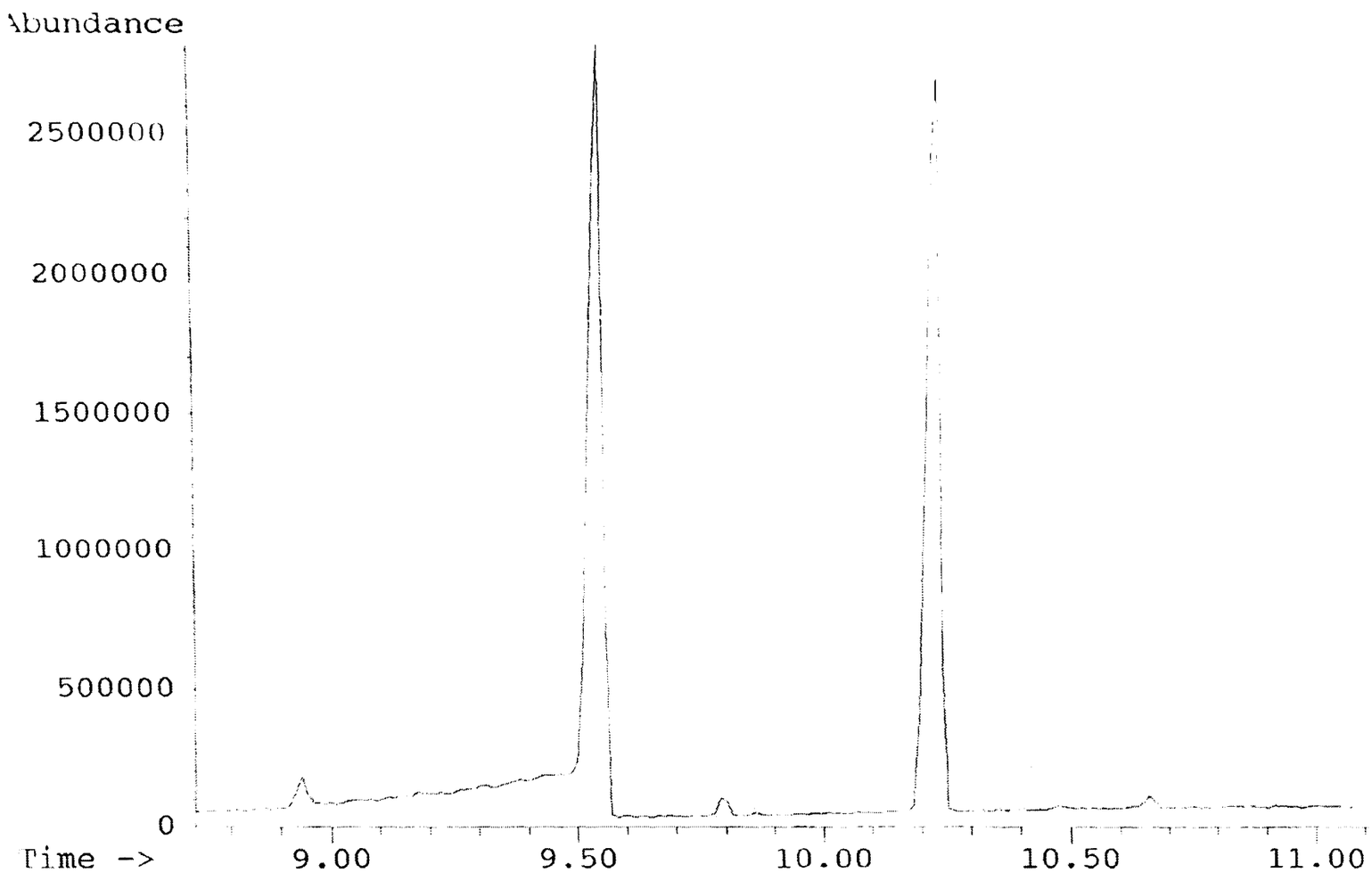


Figure 3.2. Total ion chromatogram of the tri-TBDMS derivatives of glutamate and glutamine standards.

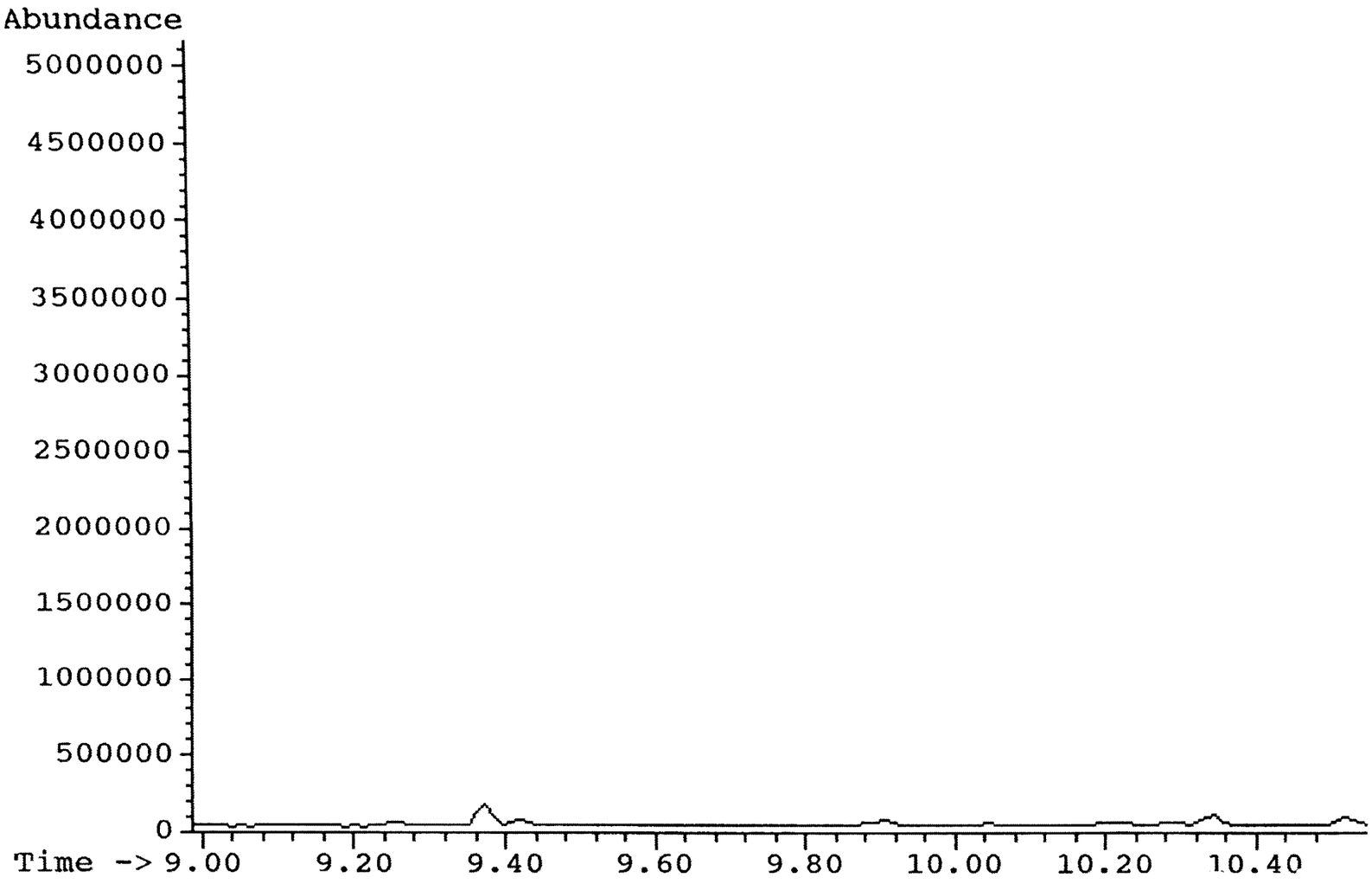


Figure 3.3. Total ion chromatogram of a derivatisation blank.

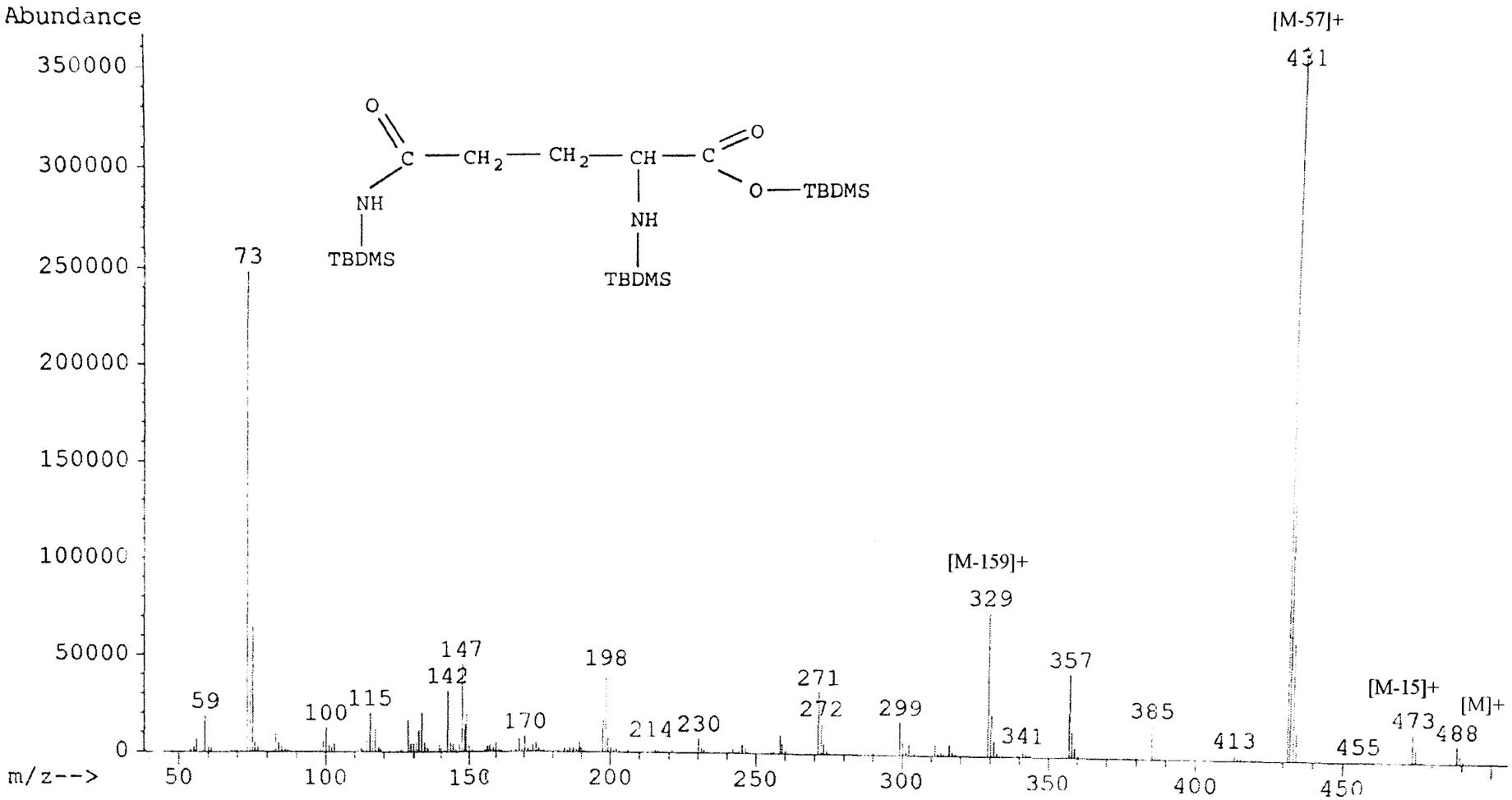


Figure 3.4. Electron impact mass spectra at 70 eV of the tri-TBDMS derivative of glutamine.

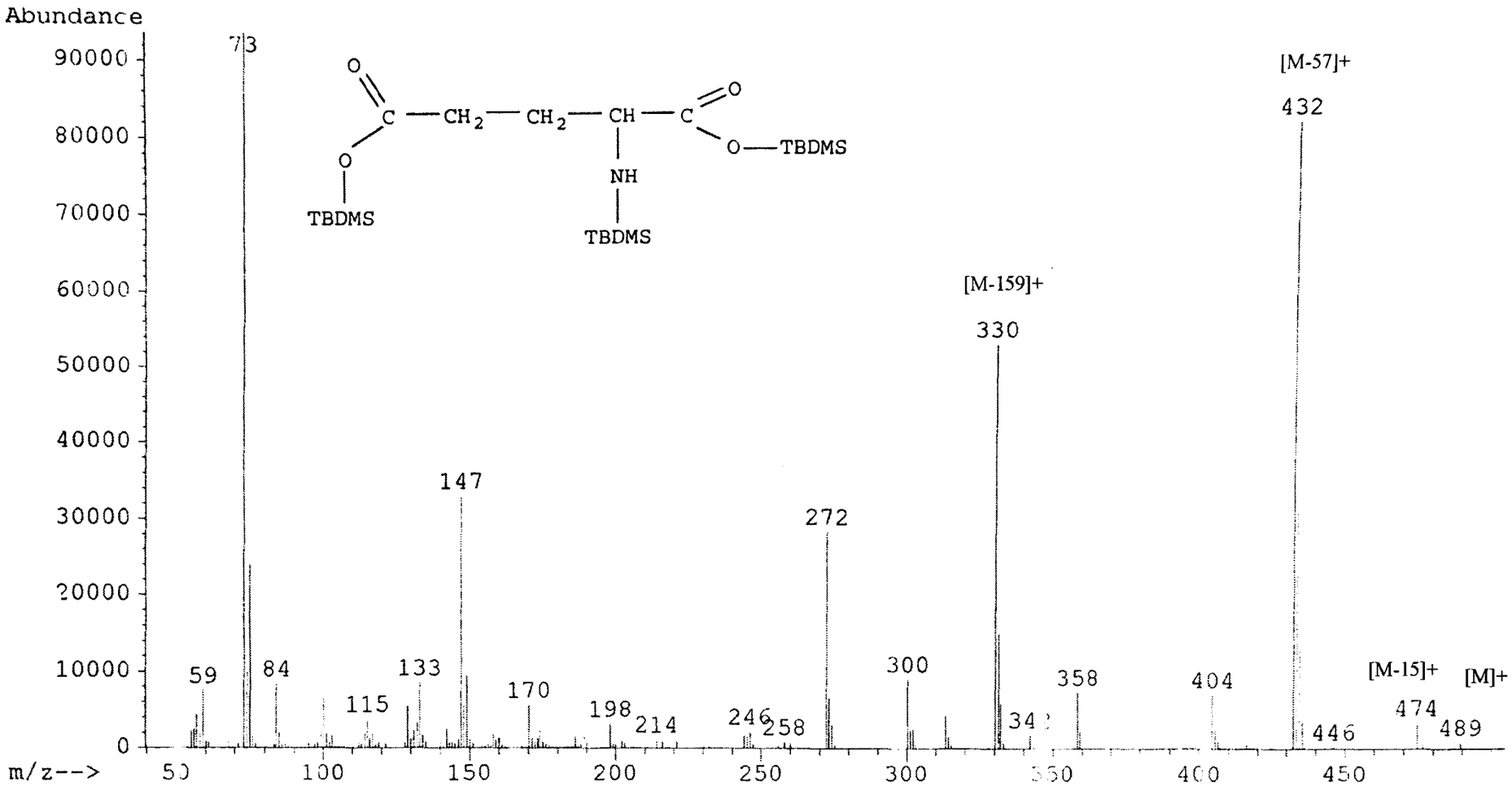


Figure 3.5. Electron impact mass spectra at 70 eV of the tri-TBDMS derivative of glutamate.

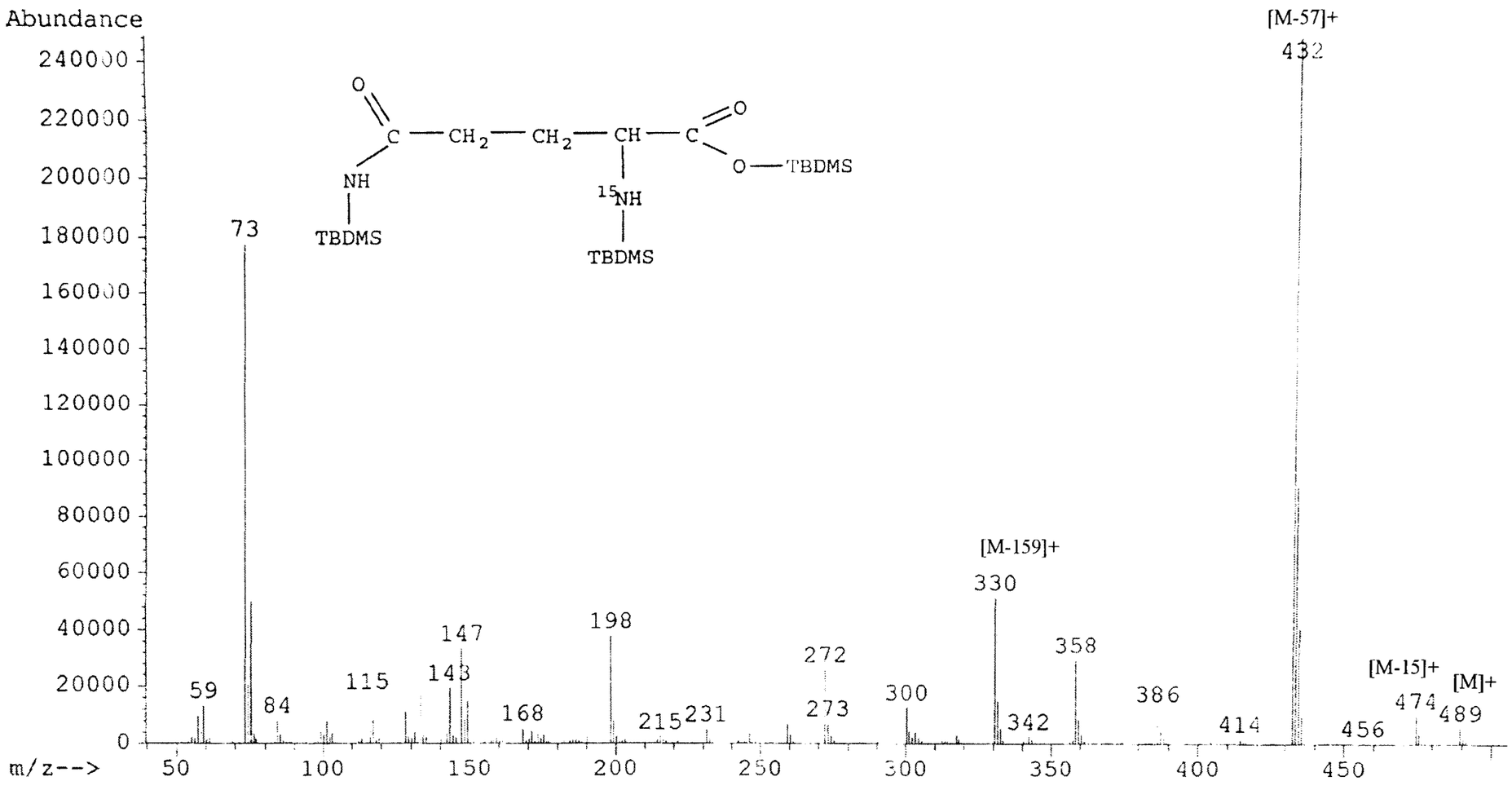


Figure 3.6. Electron impact mass spectra at 70 eV of the tri-TBDMS derivative of [2-^{15}N] glutamine.

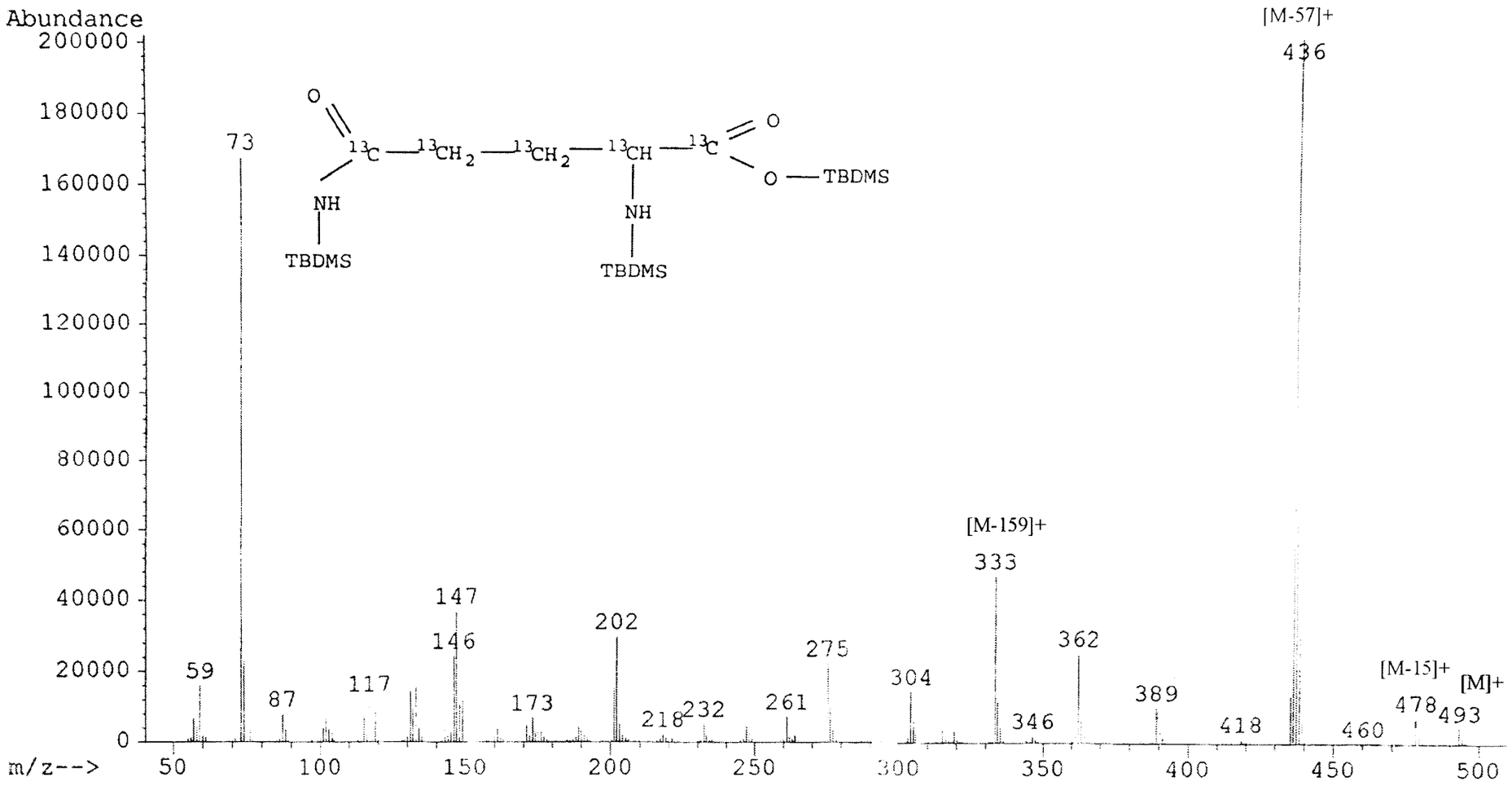


Figure 3.7. Electron impact mass spectra at 70 eV of the tri-TBDMS derivative of [U-$^{13}C_5$] glutamine.

Calculation of isotopic enrichment

The areas of the peaks at m/z 432 (M+1) and 431(M) corresponding to the labelled and unlabelled glutamine were used to calculate the enrichment (of labelled glutamine) expressed as atom percent excess (APE %)

$$\text{APE (\%)} = \frac{\text{Rs-Rb}}{(\text{Rs-Rb})+1} * 100$$

where Rs and Rb are the area ratios of (M+1)/(M) for the enriched and baseline samples, respectively.

Calibration curves

To confirm a linear response for the glutamine enrichment measurements, standards containing 0.3 μmol glutamine enriched with [^{15}N] glutamine in the range 0-10 APE were prepared and analysed on the GCMS. The observed enrichments were calculated from the ^{15}N glutamine/glutamine area ratios and plotted against the theoretical enrichments to obtain an enrichment calibration curve. A typical calibration curve is shown in Figure 3.8. A linear relationship (r^2=0.9995) was observed with a slope close to unity (1.10). The slope of the curve was used to monitor day to day instrumental drift and tuning.

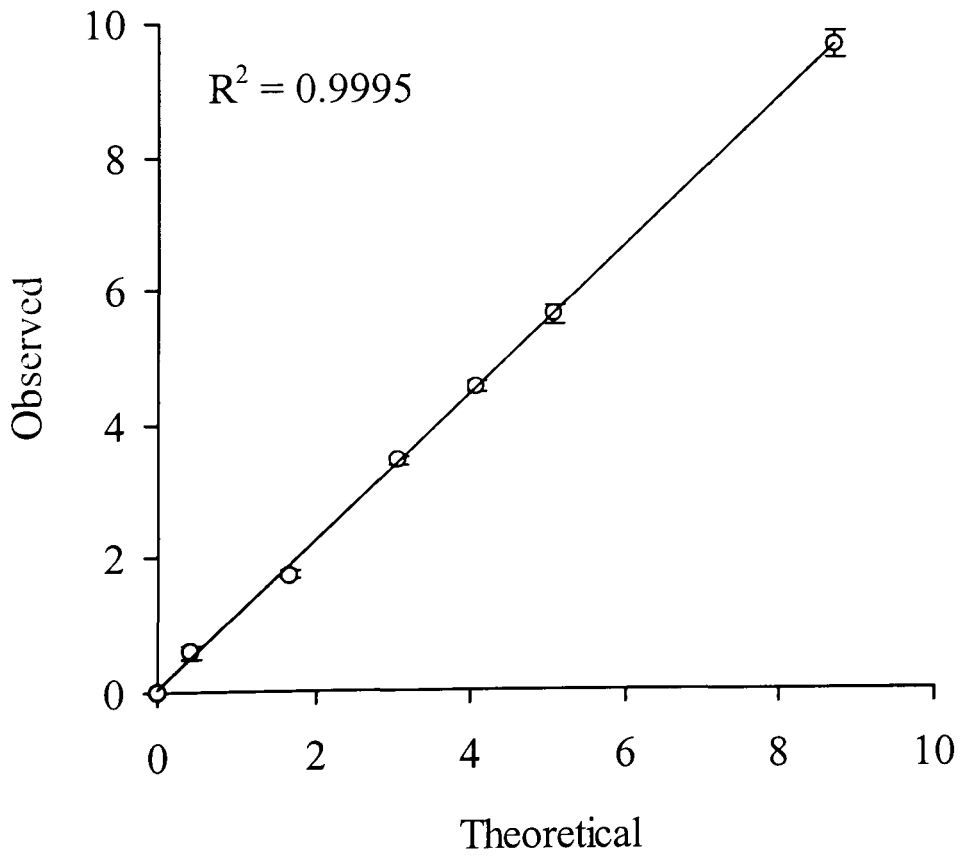


Figure 3.8. Glutamine enrichment curves (n=5), observed vs. theoretical ^{15}N enrichment (APE).

Glutamine concentrations were measured by reverse isotope dilution using [U-$^{13}C_5$] glutamine as the internal standard. Standards containing 0.1 μmol internal standard and concentrations of unlabelled glutamine in the range 0-0.5 μmol were analysed. To confirm a linear response for concentration measurements graphs of the theoretical against the observed glutamine/internal standard ratio were plotted (Figure 3.9). A correlation of 0.999 was achieved with a slope close to unity (1.07). Graphs of the observed ratio (glutamine/internal standard) against the concentration were then plotted and used to calculate the concentrations in the samples. Figure 3.10 shows the average of five concentration curves. The plasma glutamine concentrations were also recorded in plasma amino acid profiles measured on an automated amino acid analyser (Amersham Pharmacia Biotech, Bucks., UK). We were able to compare the glutamine concentrations measured on the GCMS with those measured using a different technique (Figure 3.11). A correlation of 0.8 was achieved with a slope close to unity (0.91) across a wide range of glutamine concentrations (200-800 μmol l^{-1}).

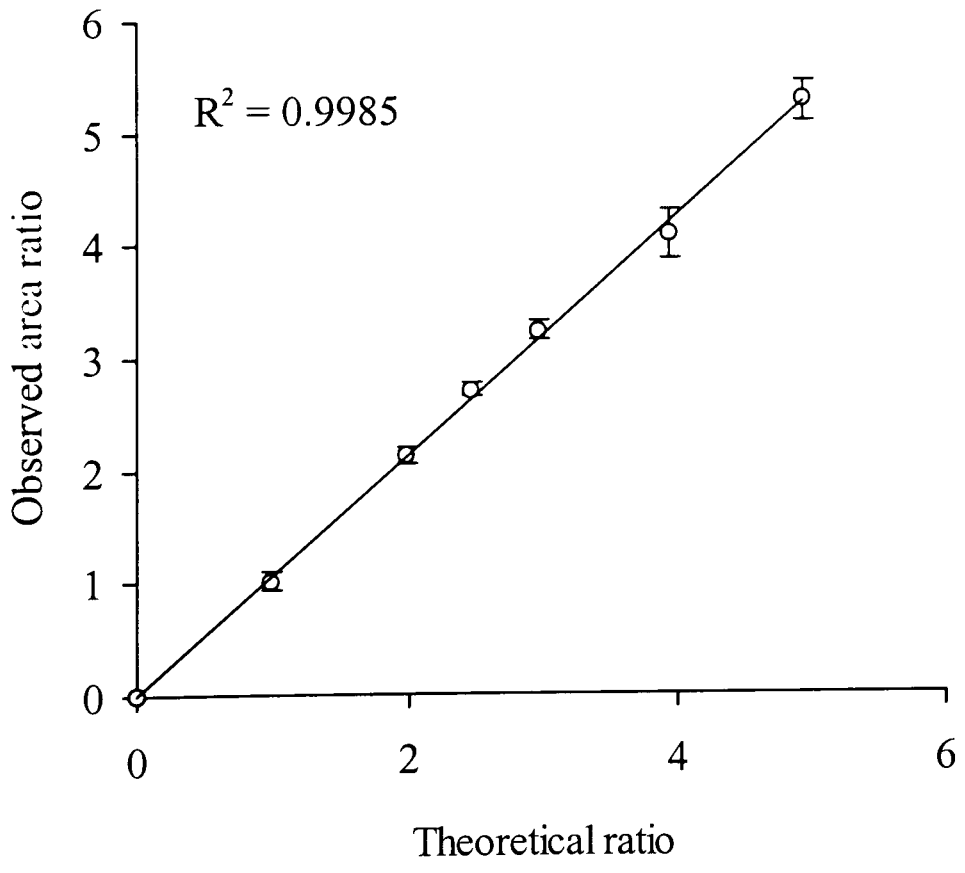


Figure 3.9. Theoretical glutamine/internal standard ratio vs. observed area ratio (n=5).

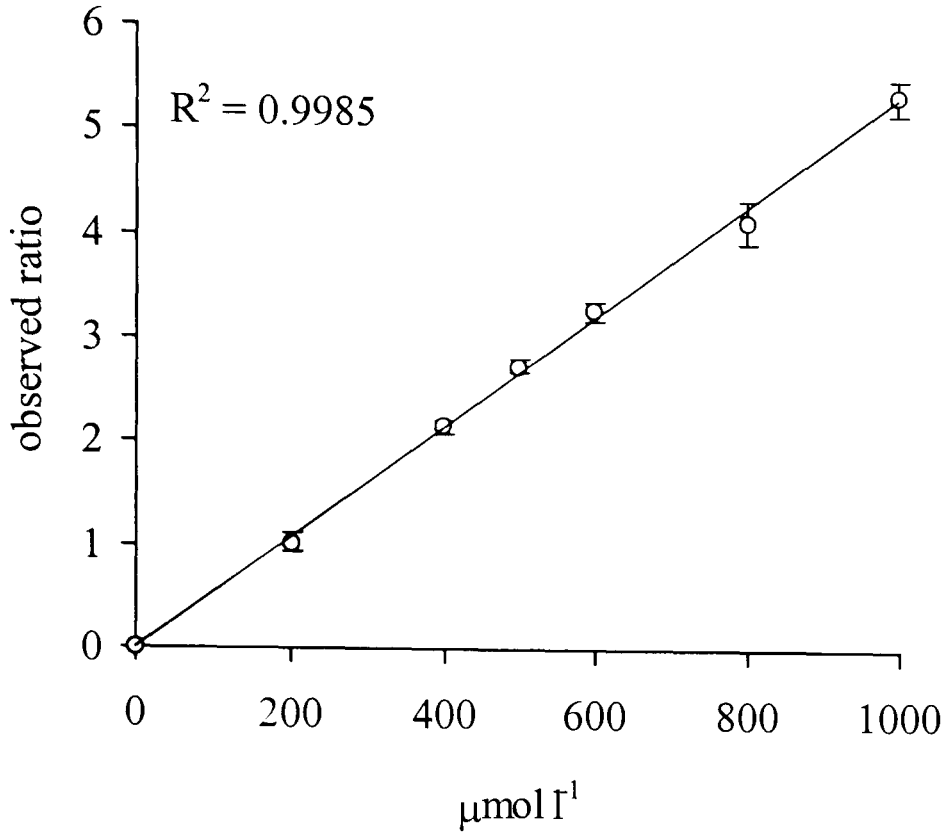


Figure 3.10. Glutamine concentration curves (n=5).

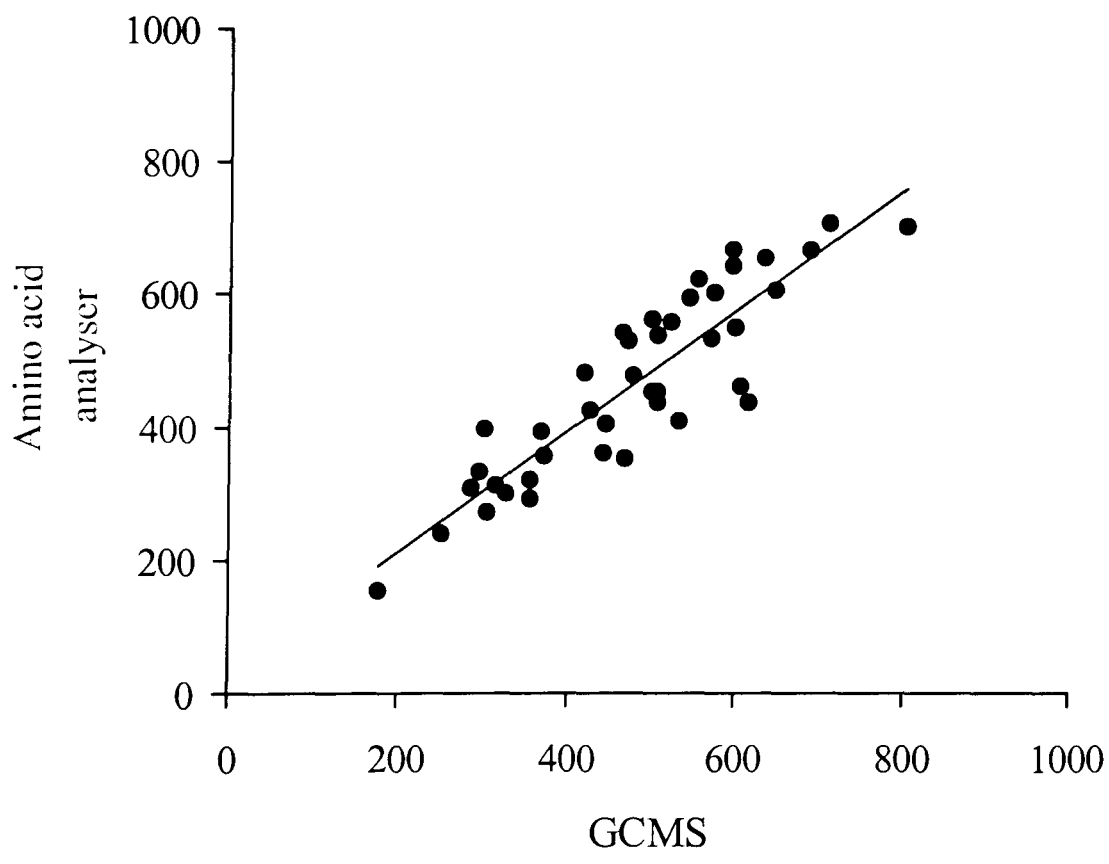


Figure 3.11. Glutamine concentration (µmol l^{-1}) measured by GCMS and the amino acid analyser.

Preparation of plasma samples

As the glutamine and glutamate standards were chromatographically separated on the GC there was no need for an additional separation step in the sample preparation procedure. A rapid cation-exchange chromatography step was chosen to isolate the amino acids from plasma samples. The final protocol (Table 3.1) was adapted from the methods of Wolfe (1992) and Nissim et al (1984). Enrichment quality control samples were made by spiking pooled plasma samples with [2-^{15}N] glutamine to give baseline and enriched (3 APE) samples. The quality controls were aliquoted and stored at -70 ^{o}C until used for assay development or with each batch of samples.

Cation exchange columns were prepared, 1.5 inches of an aqueous slurry of AG 50W-X8 resin were placed in plastic chromatography columns (Bioconnections, Leeds, UK). The columns were pre-washed with 1 M HCL to ensure that all the resin was in the H^+ form. 1 M HCL was added to the columns until pH paper indicated the eluate was pH 1 (~1ml). The columns were washed with H_2O until the eluate was neutral and were then capped to keep the resin moist until the samples were ready.

The internal standard (100nmol [U-$^{13}C_5$] glutamine) was added to 500 µl plasma aliquots during the study, before the samples were frozen. On the day of the assay the plasma samples were thawed and acidified with 500 µl, 50 % ($^{v}/_{v}$) glacial acetic acid. The acidified plasma was applied to the cation exchange column, the sample tube was washed with 1 ml H_2O and the wash transferred to the columns. The columns were washed with a further 5 ml H_2O. The amino acids were eluted by washing the column with 3M NH_4OH (4 ml), followed by 1 ml H_2O. The samples were collected once the column eluate had turned basic (as indicated by litmus paper), frozen and lyophilised overnight.

The samples were dried azeotropically with dichloromethane prior to derivatisation to remove any traces of water, as TBDMS derivatives are hydrolytically unstable. The samples were then dissolved in 100 µl acetonitrile and derivatised with 100 µl MTBSTFA at 120 ^{o}C for 2 hours. After cooling, the excess reagent was removed under N_2 at room temperature. The residue was dissolved in 600-800 µl decane

Table 3.1. Preparation of TBDMS derivative of plasma glutamine for GCMS analysis.

Column preparation

1. Prepare slurry of cation resin (AG-50W-X, H^+, 200-400 mesh) in H_2O
2. Add resin to plastic chromatography columns (~1.5 inches of settled resin).
3. Add 1M HCL (~1 ml) until pH paper indicates eluate is pH 1.
4. Wash column with H_2O (~10 ml) until pH paper indicates eluate is neutral.
5. Cap columns until samples are ready.

Procedure

1. Add 500 µl, cold 50 % acetic acid to thawed plasma sample and vortex.
2. Load sample onto columns. Allow all fluid above resin to elute before loading sample, but do not allow columns to run dry.
3. Wash sample tube with 1 ml H_2O and transfer to column.
4. Wash columns with 5 ml H_2O.
5. Elute glutamine with 4 ml 3MNH_4OH, followed by 1 ml H_2O.
6. Freeze samples and lyophilize.

Derivatisation

1. Add 500 µl dichloromethane, mix and dry sample under N_2.
2. Add 100 µl acetonitrile, 100 µl MTBSTFA, cap and mix.
3. Heat in block at 120 °C for 2 hours.
4. Remove excess reagents under N_2.
5. Add 600-800 µl decane, cap and mix.

containing 5 % MTBSTFA for GCMS analysis. Typical ion chromatograms of m/z 431, 432 and 436 from a plasma sample are shown in Figure 3.12.

To check for possible sample contamination from this sample preparation procedure saline blanks were taken through the above protocol. There were no peaks in these samples corresponding with the glutamine peak from plasma samples. In addition the area ratio measurement for unenriched plasma samples (0.376 ± 0.003, n=10) was close to the theoretical ratio (0.3748) calculated by mass spectrometer software.

Glutamine recovery from cation exchange columns was checked with [^{3}H] glutamine (250 μCi 250 μl^{-1}; Amersham Pharmacia Biotech, Bucks., UK). Ten plasma samples were spiked with tracer and taken through the above protocol. The column elutate was collected, frozen and lyophilised. The lyophilised samples were reconstituted in 1 ml H_2O, mixed with 15 mls Picofluor and counted on a liquid scintillation β counter (Rackbeta 1219, LKB/Wallac, Notts., UK). The mean value for the recovery of counts was 92 ± 0.4 % (n=10).

Using the protocol shown in Table 3.1 all the samples from each subject and the plasma quality controls were extracted in a single assay (<1 hour). A set of enrichment and concentration calibration curves were also prepared and lyophilised with the plasma samples. Each sample series (samples, quality controls and standards) was derivatised the following day and run as a single batch on the GCMS. The standards and quality controls were re-injected at the end of each sample run to check for instrument drift.

The intra-assay coefficient of variation (CV) was calculated from 10 replicates of the baseline quality control sample measured within one assay. The intra-assay CVs were 0.34 % for enrichment and 2 % for concentration (700 μmol l^{-1}). The inter-assay CV was determined for the baseline and enriched quality control samples measured in 14 separate assays over a 1 year period. The inter-assay CVs were 3 % for enrichment, and 7 % for concentration.

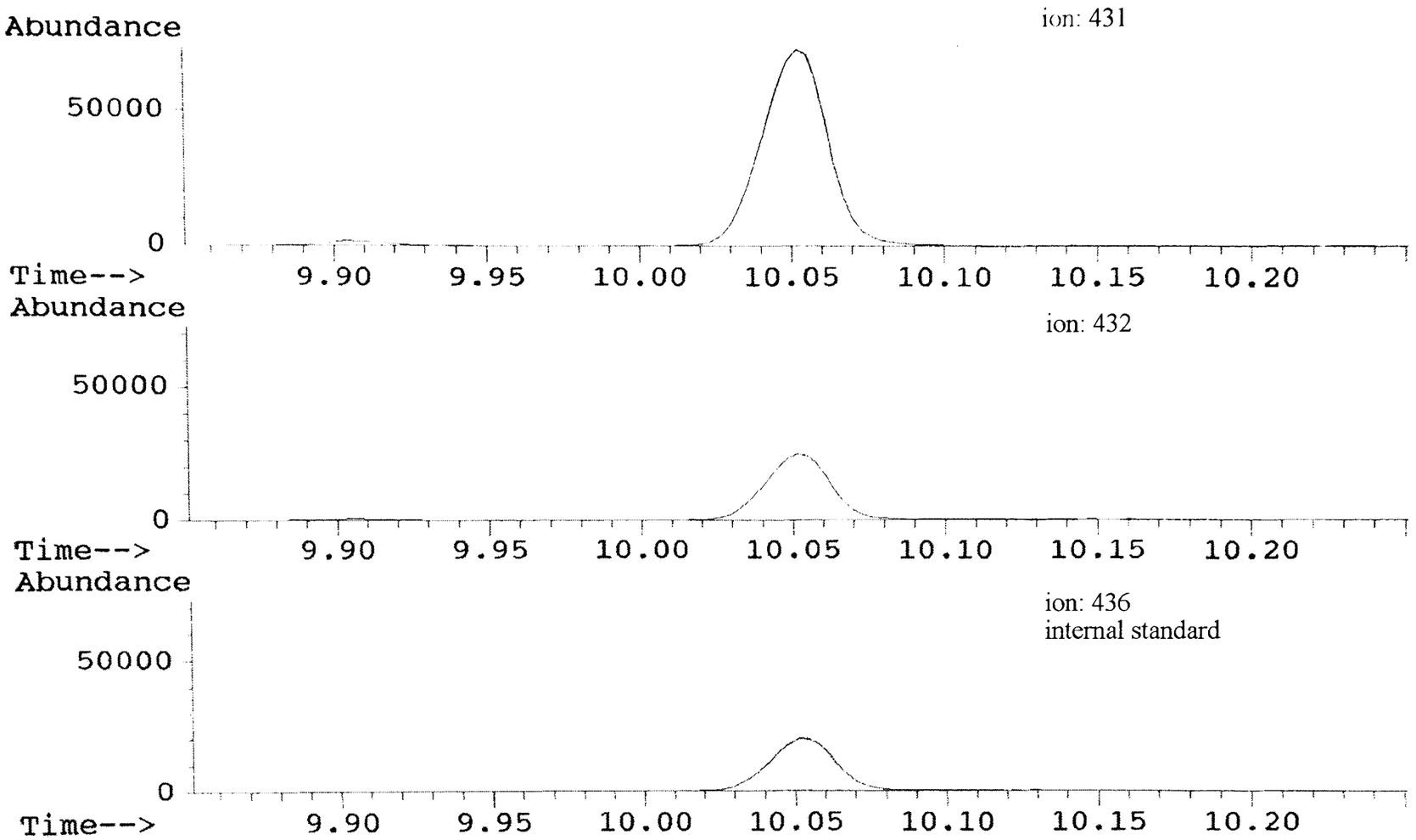


Figure 3.12. Typical selected ion chromatograms at m/z 431, 432, and 436 of the tri-TBDMS derivative of glutamine from a plasma sample representing unenriched glutamine, enriched glutamine and the internal standard.

Measurement of KIC enrichment.

Plasma KIC enrichment was used as a measure of intracellular leucine enrichment for the calculation of leucine appearance rate (see page 67) (Matthews et al 1982).

The *O*-tertiary-butyldimethylsilyl quinoxalinol derivative of KIC was prepared by a modification of the method described by Ford et al 1985. The proteins were precipitated from a 100 µl plasma aliquot with 1ml ethyl alcohol. After centrifugation the supernatant was evaporated to dryness under N_2 at 50 °C. The residue was dissolved in water (200µl) and reacted with 100 µl freshly prepared orthophenylenediamine solution (2 % in 4M HCL) at 90 °C for 1 hour. The solution was extracted twice with 1 ml ethyl acetate and the combined extracts were dried over anhydrous sodium sulphate. The solvent was evaporated at room temperature under N_2, the residue was dissolved in 100 µl acetonitrile and reacted with 100 µl MTBSTFA at 120 °C for 45 minutes. The quinoxalinol-TBDMS derivatives of the keto acids were kept in the final reaction mixture until just before analysis when the excess reagent was removed under N_2 at room temperature, and the residue dissolved in 100 µl decane containing 5 % MTBSTFA.

The derivatised keto acids were analysed on the same GCMS system described for the glutamine assay. The column temperature was held at 120 °C for 1 minute then programmed at 20 °C min^{-1} to 300 °C and held at 300 °C for 1 minute. Selected ions at m/z 259 and 260 were monitored for KIC and [1-^{13}C] KIC respectively using a dwell time of 25 msec, giving 10.64 cycles per sec. Typical ion chromatographs of m/z 259 and 260 from a plasma sample are shown in Figure 3.13. KIC enrichment was calculated using the APE equation previously described for glutamine (page 53).

Stock solutions (1mg ml^{-1}) of KIC and [1-^{13}C] KIC (98.5 %; Univar Plc., Croydon, UK) were prepared and aliquots were stored at –20 °C. On the day of the assay an enrichment calibration curve was prepared (range 0-10 APE). Ethyl alcohol (1 ml) was added to the standards which were subsequently treated the same as the plasma samples. A typical calibration curve for [1-^{13}C] KIC enrichment is shown in Figure 3.14. A linear relationship (r^2=0 .9998) was observed over the working range of

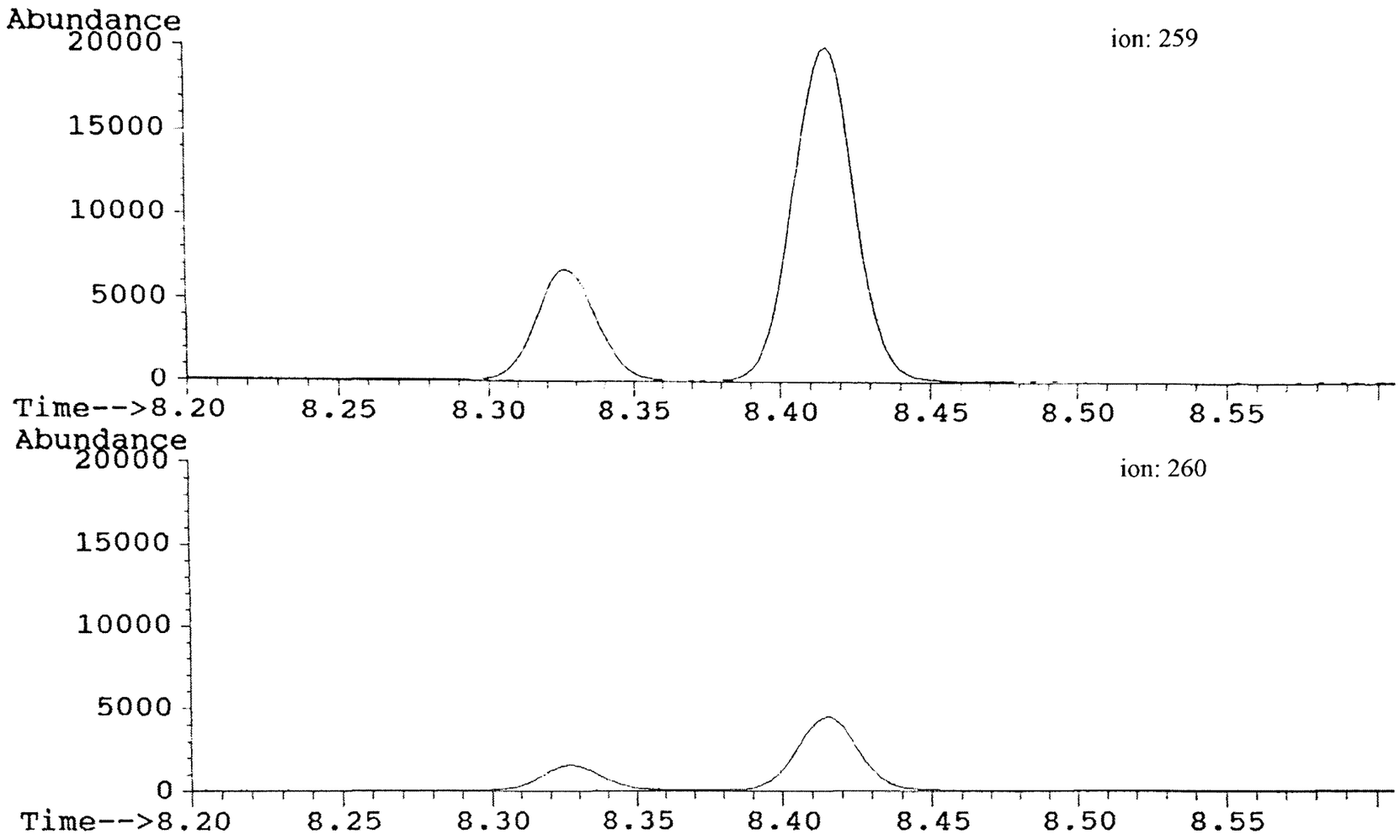


Figure 3.13. Typical selected ion chromatograms at m/z 259 and 260 of the quinoxalinol-TBDMS derivative of KIC from a plasma sample representing unenriched and enriched KIC (peak at 8.42 minutes).

0-10 APE with a slope almost equal to unity (0.9977). The enrichment standard curve was run at the beginning and end of each sample run to check for instrumental drift across the sample run. Enrichment quality controls were prepared by spiking plasma with [1-^{13}C] KIC to give baseline, medium (~3 APE) and high (~6.5 APE) enriched samples. The inter-assay coefficient of variations were 1.86, 1.15 and 1.21 % respectively (n=10).

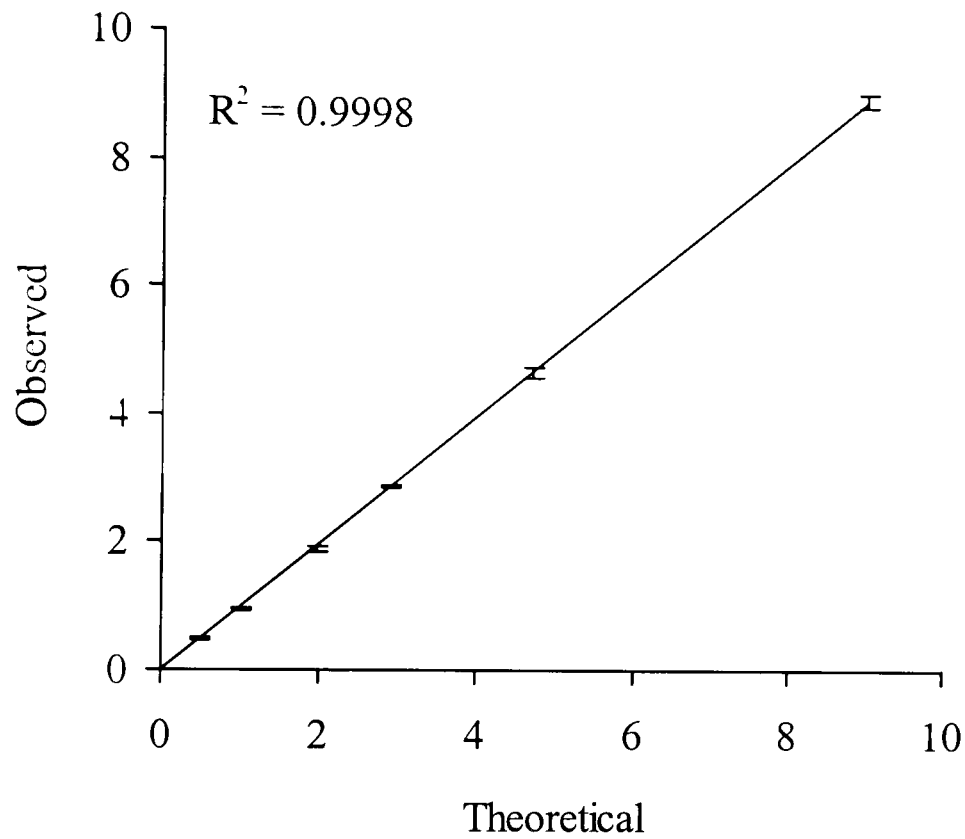


Figure 3.14. KIC enrichment curves (n=5) of observed vs. theoretical ^{13}C KIC enrichment (APE).

Metabolite assays

Glucose

Plasma glucose concentrations were measured on a Clandon model 23AM Glucose analyser (Yellow Springs Instruments, Hamps, UK). This instrument utilises glucose oxidase, immobilised between two membranes, coupled with a hydrogen peroxide sensor. A constant volume of standard or plasma (25 µl) is introduced a reaction chamber using a syringe-pipette. The glucose diffuses through a membrane into contact with the enzyme glucose oxidase which catalyses the reaction:

$$\text{D-glucose} + O_2 \longrightarrow \text{gluconic acid} + H_2O_2$$

The H_2O_2 generated diffuses through a second membrane which excludes glucose, to a platinum anode and a constant proportion is oxidised. The current created is directly proportional the glucose concentration in the sample:

$$H_2O_2 \longrightarrow 2H^+ + O_2 + 2e^-$$

A silver cathode at which O_2 is reduced to water completes the circuit:

$$4H^+ + O_2 \longrightarrow H_2O + 2e^-$$

At the start of each analysis the instrument was calibrated using 0, 5.6, 10, and 25 mmol l^{-1} glucose standard solutions. During analysis the calibration was checked with the 10 mmol l^{-1} standard every 10 samples. If the results differed by more than 0.2 mmol the calibration was repeated.

Amino acids

Plasma amino acids concentrations were measured using an Alpha plus series II automated amino acid analyser (Amersham Pharmacia Biotech , Bucks., UK) which uses cation-exchange chromatography and post-column derivatisation with ninhydrin to separate and detect the amino acids.

The stored amino acid samples were thawed, the pH adjusted to 2.2 if necessary and filtered (0.2 μm). The amino acids are separated on a sulphonated polystyrene resin (Ultropac 8) using a system of 5 lithium based buffers of varying pH and ionic strength. Controlling the column temperature enhances the chromatography. The post-column ninhydrin reaction proceeds in the reaction coil at 135 °C where the amino acids react to form chromophores with maximum absorption at 570 or 440 nm. The light absorption is measured at these two wavelengths in the photometer unit which is connected to an integrator (Spectra-physics SP4270: Thermo Separation Products, Staffs, UK). All buffers and reagents for the amino acid analyser were purchased from Amersham Pharmacia Biotech.

The amino acid is identified by its retention time relative to a standard and the area under the peak indicates concentration when compared to the internal standard (nor-leucine). An amino acid standard solution, with added glutamine and ornithine, was prepared at 0.1 μmol l^{-1} (A-2908; Sigma-Aldrich, Dorset, UK) and standards were run every 6-8 samples. To correct for differences in the "colour yields" for the reactions of

the different amino acids with ninhydrin, a colour index for each amino acid is calculated from the standard as follows:

$$\text{Colour Index (CI)} = \frac{\text{area nor-leucine peak}}{\text{area amino acid peak}}$$

The average CI was calculated for a set of samples between two standards, the amino acid concentrations were then calculated as follows:

$$[\text{amino acid}]\ (\mu\text{mol l}^{-1}) = \frac{\text{area nor-leucine peak}}{\text{area amino acid peak}} * \text{CI} * \text{dilution factors}$$

Infusate glutamine and leucine concentrations were also measured on the amino acid analyser. An aliquot of the infusate was diluted (x 250) with lithium citrate buffer (pH 2.2) and then mixed with an equal volume of 10 % sulphosalicylic acid containing the internal standard nor-leucine (100 nm ml^{-1}). The pH was adjusted to 2.2 and the sample filtered through 0.2 μm syringe filters before transferring the sample to the analyser. Inter and intra assay CVs for amino acid concentrations are shown in Table 3.2.

Hormone assays

Serum insulin concentrations were measured in duplicate, using an in-house double antibody radioimmunoassay described by Sönksen (1976). Briefly, 50 μl aliquots of serum were incubated overnight, at room temperature with the first antibody, guinea pig anti-insulin serum (GP8) and the tracer, ^{125}I-A^{14}-tyr-monoiodo-insulin (Amersham Pharmacia Biotech , Bucks., UK). 100 μl of the second antibody, a solid phase sheep-anti-guinea pig serum antibody (Pharmacia Decanting Suspension 1) was added and the samples were incubated for a further 2 hours. 1 ml of water was added to the sample immediately prior to centrifugation (1500 g, 4 °C, 45 minutes), the supernatant immediately decanted and the precipitate counted on the gamma counter (Gammamaster 1277, LKB/Wallac). The RiaCalc programme (Amersham Pharmacia Biotech) was used to determine the sample concentrations from the standard curve. The standard curve range was 0-1000 mU l^{-1} and the assay gave a normal fasting range of $\leq$ 12 mU l^{-1}. The intra and inter assay CVs were 6 and 9 % respectively.

Table 3.2. Intra and inter assay CVs for plasma amino acid concentrations

Amino Acid	*Intra assay (n=10)*	*Inter assay (n=10)*
Aspartate	33	25
Threonine	5	4
Serine	5	4
Glutamate	5	11
Glutamine	5	6
Glycine	5	4
Alanine	5	4
Valine	4	4
Cystine	5	4
Methionine	8	7
Isoleucine	4	17
Leucine	4	5
Tyrosine	15	16
Phenylalanine	7	5
Ornithine	6	6
Lysine	5	4
Histidine	5	5

C-peptide concentrations were measured using a similar in-house double antibody radioimmunoassay. The first antibody, guinea pig anti-C-peptide serum, was purchased from Biogenesis Ltd. (Poole, UK). The tracer ^{125}I-Tryosyl-C-peptide was donated by Dr A. Lindsey (Hammersmith Hospital). The second antibody was Pharmacia Decanting Suspension as above. The standard curve range was 0-3.3 nmol l^{-1} and the assay gave a normal fasting range of 0.25-0.33 nmol l^{-1} (20-30 years). The intra and inter CVs were 9 and 11 % respectively.

Glucagon levels were also determined using similar methodology, but using a commercially available radioimmunoassay kit (Biogenesis Ltd, Poole, UK). All the samples from this study were assayed using a single kit. The ICU patients' samples were diluted according to expectation. The standard curve range was 0-100 pg ml^{-1}, and the assay gave a normal fasting range of 50-150 pg ml^{-1}. The intra assay CV was <10 %.

GH levels were measured using a commercially available immunoradiometric assay kit (Skybio Ltd., Beds, UK). The GH assay is a two-site immunoradiometric assay. The first antibody is [^{125}I]-labelled for detection, the second is coupled to solid phase for separation. Unbound materials are removed by centifugation and washing the tubes. The standard curve range was 0-200 mU l^{-1}. Undetectably low values were considered as concentrations at the detection limit of the assay. All the samples from this study were assayed using a single kit, the intra assay CV was <2 %.

IGFBP-1 and 3 concentrations were measured using coated-tube immunoradiometric assay kits (Diagnostic Systems Laboratories, Texas, USA). These kits employ a two-site immunoradiometric assay in which the IGFBP-1 (or 3) is sandwiched between two antibodies. The first antibody is immobilised to the inside walls of the assay tubes, the second is radiolabelled for detection. Unbound materials are removed by decanting and washing the tubes. The standard curve range for the IGFBP-1 assay was 0-160 ng ml^{-1}, samples outside the standard curve range were diluted with the appropriate buffer and reassayed. The standard curve range for the IGFBP-3 assay was 0-100 ng ml^{-1}, all samples were diluted 1:100 with the appropriate buffer before analysis. The intra and inter assay CVs were <1 and <6 % for IGFBP-1 and <4 and <1 % for IGFBP-3 respectively.

Cortisol, thyroid hormones, and IGF-I concentrations were all measured by immunoassay in the Chemical Pathology Department at St Thomas' Hospital. Cortisol was measured by an enzyme linked immunosorbent assay using the Enzymun-Test cortisol kit (Boehringer Mannheim, Sussex, UK). Free T_4 and T_3 were measured by solid phase competitive immunoassays using chemiluminesence (Chiron Diagnostics, Essex, UK). Total IGF-I was measured by an in-house radioimmunoassay after acid ethanol precipitation of the IGFBPs.

Calculations

Calculation of glutamine kinetics

Measurements of glutamine metabolism were calculated using standard isotope dilution equations. Glutamine appearance rate (Ra_{gln}; μmol min^{-1} kg^{-1}) was calculated as:

$$Ra_{gln} = F\,(1 / (APE_{gln} * 0.01) - 1)$$

where F is the isotope infusion rate (μmol min^{-1} kg^{-1}) and APE_{gln} is the steady state plasma [2-^{15}N]glutamine enrichment. In the post-absorptive state the endogenous rate of glutamine appearance in plasma (Endo Ra_{gln}) is equal to the calculated Ra_{gln}. At steady state, the rate of glutamine disappearance from plasma (glutamine uptake: Rd_{gln}) was assumed to be equal to Ra_{gln}. Glutamine metabolic clearance rate (MCR_{gln}; ml min^{-1} kg^{-1}) was calculated as:

$$MCR_{gln} = Rd_{gln} / [gln]$$

where [gln] is the steady state plasma concentration of glutamine (μmol l^{-1}).

Leucine Ra was calculated in a similar manner using the plasma enrichment of KIC as an estimate of the intracellular leucine enrichment (Matthews et al 1982). Whole body leucine kinetics can be determined by a short, continuous infusion of [1-^{13}C] leucine and measurement of plasma ^{13}C leucine enrichment (Matthews et al 1980). The plasma leucine enrichment is higher than the intracellular leucine enrichment because 1) the leucine tracer is infused directly into the extracellular, plasma compartment and 2) unlabelled leucine from protein breakdown dilutes the leucine enrichment in the intracellular compartment (Matthews et al 1982). KIC is formed intracellularly from the transamination of intracellular leucine and is released

into the systemic circulation. Plasma KIC enrichment is therefore assumed to be a more valid indicator of the intracellular leucine enrichment.

Estimation of glutamine de novo synthesis

The whole body turnover (equal to Ra at steady state) of an essential amino acid such as leucine (Q_{leu}) can be described by the following equation

$$Q_{leu} = S_{leu} + C_{leu} = B_{leu} + I_{leu}$$

where S_{leu} is the incorporation of leucine into body proteins (protein synthesis), C_{leu} is leucine catabolism, B_{leu} is the release of leucine from body protein (proteolysis) and I_{leu} is the dietary intake of leucine. In the post absorptive state I_{leu} is negligible and so

$$Q_{leu} = B_{leu}$$

For a non-essential amino acid (glutamine) an additional term is added to the above model, D_{gln} which represents the de novo synthesis of glutamine

$$Q_{gln} = S_{gln} + C_{gln} = B_{gln} + D_{gln} + I_{gln}$$

In the post absorptive state there are only two inflow components to the turnover of a non-essential amino acid

$$Q_{gln} = B_{gln} + D_{gln}$$

Assuming amino acids are released from protein at rates proportional to their concentration in body protein, B_{gln} can be estimated as follows

$$B_{gln} = k * B_{leu}$$

where k is the assumed molar ratio of glutamine to leucine content of body protein. Body protein contains 13.9 g of glutamine and glutamate per 100g, but the exact glutamine content of body protein is not known so glutamine is assumed to contribute half the total content (7 g or 47.9 mmol). 100g of body protein contains 8 g or 61.1 mmol leucine. This gives a k value of 0.78 (i.e. 47.9/61.1) for the molar ratio of glutamine to leucine in body protein (Darmaun et al 1998). The rate of de novo glutamine synthesis can then be estimated as

$$D_{gln} = Ra_{gln} - B_{gln}$$

Nutritional support was maintained throughout the second study in the critically ill patients. The above equations were corrected for the known exogenous rates of leucine and glutamine infusion. In all three treatment groups the leucine turnover has two inflow components: exogenous leucine intake (I_{leu}) from the TPN and protein

breakdown (B_{leu}). I_{leu} can be calculated from the TPN regime and therefore B_{leu} can be estimated as

$$B_{leu} = Q_{leu} - I_{leu}$$

In the patients recieving TPN the fraction of glutamine turnover ($Q_{gln} - B_{gln}$) not accounted for by proteolysis arises from de novo synthesis. In the patients recieving TPNGLN this fraction can arise either from de novo synthesis or from the exogenous glutamine intake

$$Q_{gln} = B_{gln} + D_{gln} + I_{gln}$$

I_{gln} can be calculated from the TPNGLN regime and therefore D_{gln} can be estimated.

Statistics

Results are presented as means ± standard error of means (SE). Steady state for plasma glutamine and KIC enrichment was confirmed as an insignificant correlation with time ($p>0.05$) by repeated-measures ANOVA (NCSS 6.0, Dr J. Hintze, Utah, USA). Comparisons between Study 1 and Study 2 in the critically ill patients were made by paired *t* tests. All other comparisons between groups were made by unpaired *t* tests using equal or unequal variance as necessary. The insulin, GH, cortisol and glucagon data were log transformed before analysis due to non-normal distribution.

Chapter 4
GLUTAMINE METABOLISM IN NORMAL SUBJECTS

Introduction

Stable isotope tracers have been used to investigate the kinetics of a number of amino acids in healthy humans. The amino acid glutamine has attracted considerable interest as it is an important intermediate in a number of metabolic pathways and plays a key role in nitrogen homeostasis. However, to date there have been no published reports of studies using stable isotope tracer techniques to investigate the effect of age on glutamine metabolism in normal adults.

Aim

The aim of this study was to establish a method using L-[2-^{15}N] glutamine as a tracer to investigate whole body glutamine metabolism in normal adult subjects and to compare glutamine metabolism in young and elderly healthy subjects.

Subjects

Twelve healthy volunteers were recruited from staff and their relatives of the Department of Diabetes, Endocrinology and Internal Medicine, St Thomas' campus. All were in good general health. There was no recent relevant medical history and none were on any regular medication. The study protocol is described in Chapter 2.

Results

The physical characteristics of the normal subjects are shown in Table 4.1. The twelve normal subjects studied were divided into two groups based on their ages, both groups contained three males and three females. The elderly (>60 years) group had a mean age of 68 ± 2 years, whereas the young (<35 years) group had a mean age of 28 ± 2 years. The mean weights of the groups were 80.3 ± 4.2 and 69.8 ± 3.0 kg, respectively ($p=0.068$). Body mass index (BMI) and fat mass were significantly higher in the elderly group 28.1 ± 1.6 vs. 23.2 ± 0.4 kg m^{-2} ($p<0.05$) and 28.8 ± 4.7 vs. 17.1 ± 2.2 kg ($p<0.05$), respectively. LBM was not significantly different between the two groups 51.5 ± 6.3 vs. 52.7 ± 4.6 kg.

Table 4.1. Physical characteristics of normal subjects.

Subject No.	*Gender*	*Age (yrs)*	*Weight (kg)*	*Height (cm)*	*BMI (kg m^{-2})*	*LBM (kg)*	*Fat Mass (kg)*
young	**(<35 years)**						
1	F	31	65.4	167	23.5	47.2	18.2
2	M	27	80.7	184	23.8	68.5	11.9
3	M	23	76.7	183	22.9	65.8	10.9
4	F	25	66.0	166	24.0	45.7	20.3
5	F	29	62.0	171	21.2	44.8	17.2
6	M	34	68.0	170	23.5	44.1	23.9
mean ± SE	**3M:3F**	**28 ± 2**	**69.8 ± 3.0**	**174 ± 3**	**23.2 ± 0.4**	**52.7 ± 4.6**	**17.1 ± 2.2**
elderly	**(>60 years)**						
7	F	65	78.9	158	31.8	41.5	37.4
8	M	70	91.5	174	30.2	62.8	28.7
9	F	67	76.2	156	31.3	29.5	46.7
10	M	65	86.6	185	25.3	69.2	17.4
11	M	77	85.5	174	28.2	62.0	23.5
12	F	62	62.8	170	21.7	44.0	18.8
mean ± SE	**3M:3F**	**68 ± 2**	**80.3 ± 4.2**	**169 ± 5**	**28.1 ± 1.6**	**51.5 ± 6.3**	**28.8 ± 4.7**

BMI, body mass index; LBM, lean body mass.

Metabolite and hormone profiles are shown in Table 4.2. Plasma glucose levels were significantly higher in the elderly volunteers ($p<0.05$). There was no difference in insulin, C-peptide or GH levels between the two groups. IGF-I levels were significantly lower ($p<0.05$) in the elderly group, but there were no differences in IGFBP-1 or 3 levels. Cortisol levels were significantly higher ($p<0.05$) in the elderly group, but there were no differences in glucagon or thyroid hormone levels.

Table 4.3 shows that plasma amino acid profiles were similar in both groups of normal volunteers, with the exception of decreased serine ($p<0.01$) and histidine ($p<0.05$) and increased cystine ($p<0.01$) concentrations in the elderly subjects.

Table 4.2. Hormone and metabolite concentrations of young (<35) and elderly (>60) normals. Values are means ± SE, *n=6*.

	Units	***Young Normals***	***Elderly Normals***	***p***
Glucose	mmol l^{-1}	5.1 ± 0.1	5.8 ± 0.2	< 0.05
Insulin	mU l^{-1}	7.0 ± 1.2	11.9 ± 2.7	NS
C-peptide	nmol l^{-1}	0.28 ± 0.03	0.48 ± 0.13	NS
GH	mU l^{-1}	5.96 ± 2.49	4.44 ± 1.21	NS
IGF-I	nmol l^{-1}	22.3 ± 1.1	16.5 ± 1.7	< 0.05
IGFBP-1	ng ml^{-1}	42 ± 5	35 ± 4	NS
IGFBP-3	ng ml^{-1}	2889 ± 310	3254 ± 325	NS
Cortisol	nmol l^{-1}	205 ± 16	342 ± 40	< 0.05
Glucagon	pg ml^{-1}	61 ± 5	64 ± 6	NS
Free Thyroxine	pmol l^{-1}	14.8 ± 0.4	14.7 ± 0.6	NS
Free Tri-iodothyronine	pmol l^{-1}	4.0 ± 0.4	4.6 ± 0.2	NS

Table 4.3. Plasma amino acid profiles of young (<35) and elderly (>60) normals. Values are means ± SE, *n=6*.

Amino acid (μmol l⁻¹)	*Young Normals*	*Elderly Normals*	*p*
Aspartate	6 ± 1	6 ± 1	NS
Threonine	121 ± 7	120 ± 21	NS
Serine	115 ± 6	91 ± 5	< 0.01
Glutamate	44 ± 6	61 ± 10	NS
Glutamine	581 ± 35	534 ± 23	NS
Glycine	204 ± 9	202 ± 22	NS
Alanine	216 ± 20	283 ± 25	NS
Valine	198 ± 17	215 ± 18	NS
Cystine	50 ± 1	68 ± 4	< 0.01
Methionine	18 ± 1	33 ± 11	NS
Isoleucine	42 ± 4	36 ± 6	NS
Leucine	125 ± 12	134 ± 9	NS
Tyrosine	38 ± 4	39 ± 9	NS
Phenylalanine	41 ± 3	44 ± 5	NS
Ornithine	42 ± 4	46 ± 4	NS
Lysine	144 ± 10	166 ± 17	NS
Histidine	83 ± 8	63 ± 4	< 0.05
BCAA	365 ± 31	385 ± 32	NS
Total	2150 ± 72	2241 ± 114	NS

BCAA, branched chain amino acids.

In five of the young normals we took extra blood samples to monitor the rise in plasma glutamine enrichment to steady state and the fall in enrichment once the tracer infusion had been stopped. This allowed us to determine that a 4 hour infusion protocol achieved a "steady state" of plasma glutamine enrichment and to calculate the distribution volume of the infused glutamine. The time course of the plasma glutamine enrichments in the young normals shows a "steady state" was achieved at 4 hours (Figure 4.1). An unprimed, four hour constant infusion protocol was therefore used for the remainder of the studies. Using this data and a single pool compartmental model (SAAM II, University of Washington, 1994) we estimated a tracer miscible pool of 256 ± 15 μmol kg^{-1}.

Figure 4.2 shows the plasma glutamine enrichments and concentrations for the two groups of normal subjects during the final 30 minutes of tracer infusion indicating that "steady state" was achieved. There was no difference in plasma glutamine concentration between the two groups of normals (534 ± 23 vs. 581 ± 35 μmol l^{-1}, elderly vs. young) (Figure 4.3). Ra_{gln} was significantly lower in the elderly group (4.15 ± 0.33 vs. 5.20 ± 0.22 μmol min^{-1} kg^{-1}; $p<0.05$), but although MCR_{gln} was lower in the elderly group this did not reach statistical significance (7.77 ± 0.55 vs. 9.03 ± 0.4 ml min^{-1} kg^{-1}; $p=0.09$) (Figure 4.3). The estimated rate of release of glutamine from protein breakdown was similar in both groups of control subjects (1.47 ± 0.13 vs. 1.34 ± 0.09 μmol min^{-1} kg^{-1}), but the rate of glutamine appearance from de novo synthesis was significantly lower in the elderly group (3.57 ± 0.09 vs. 2.81 ± 0.28 μmol min^{-1} kg^{-1}, $p<0.05$). There was no difference in the proportion of Ra_{gln} arising from de novo glutamine synthesis (67 ± 2 vs. 71 ± 2 %) or protein breakdown (33 ± 2 vs. 29 ± 2 %) in the two groups (Figure 4.4).

However, when the results were expressed per kg lean body mass (Figure 4.5), the difference in Ra_{gln} (6.96 ± 0.37 vs. 6.69 ± 0.51 μmol min^{-1} kg LBM^{-1}) and the difference in the rate of glutamine appearance from de novo synthesis were no longer evident (4.77 ± 0.21 vs. 4.47 ± 0.29 μmol min^{-1} kg LBM^{-1}).

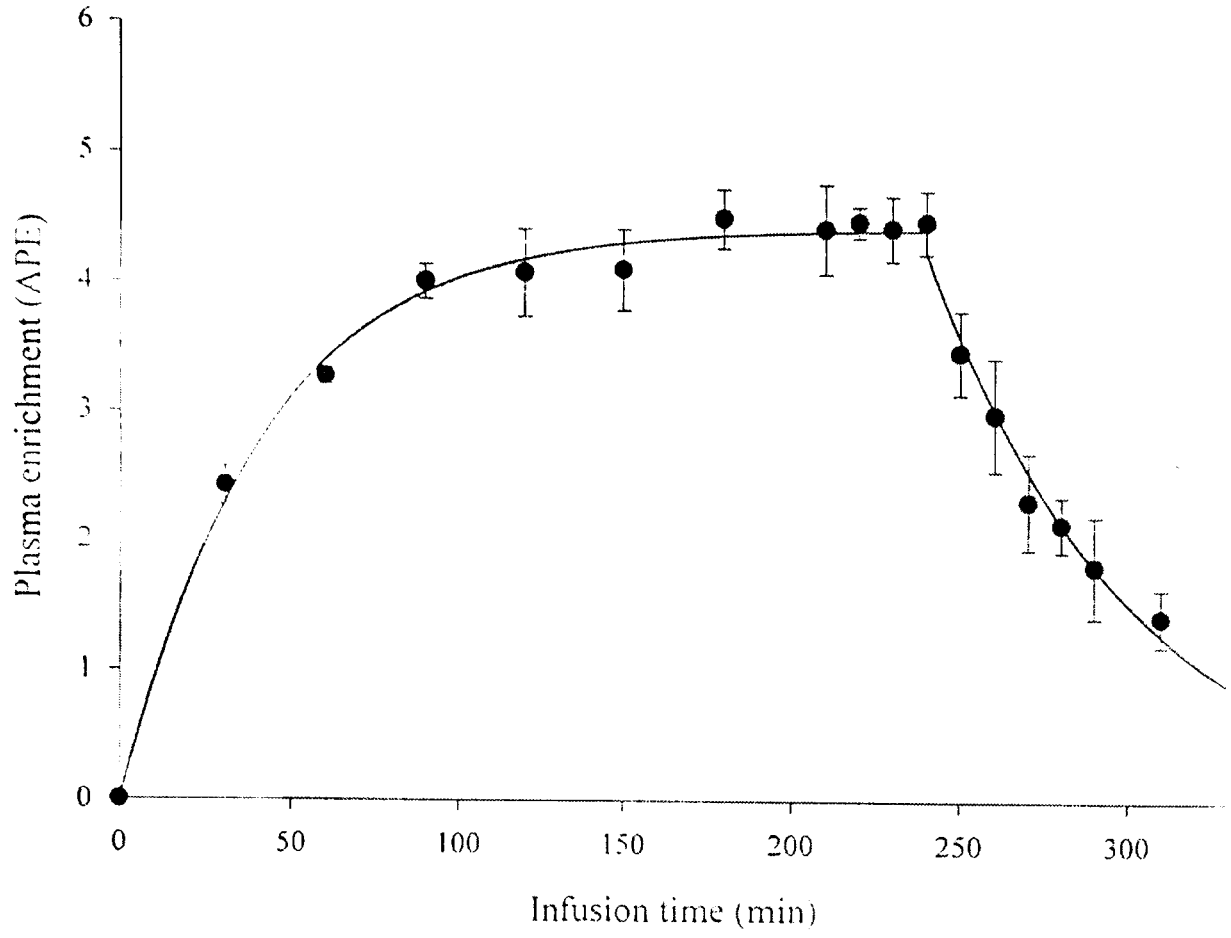


Figure 4.1. Time course of the plasma glutamine enrichments (APE) in young normals. Values are mean ± SE, n=5.

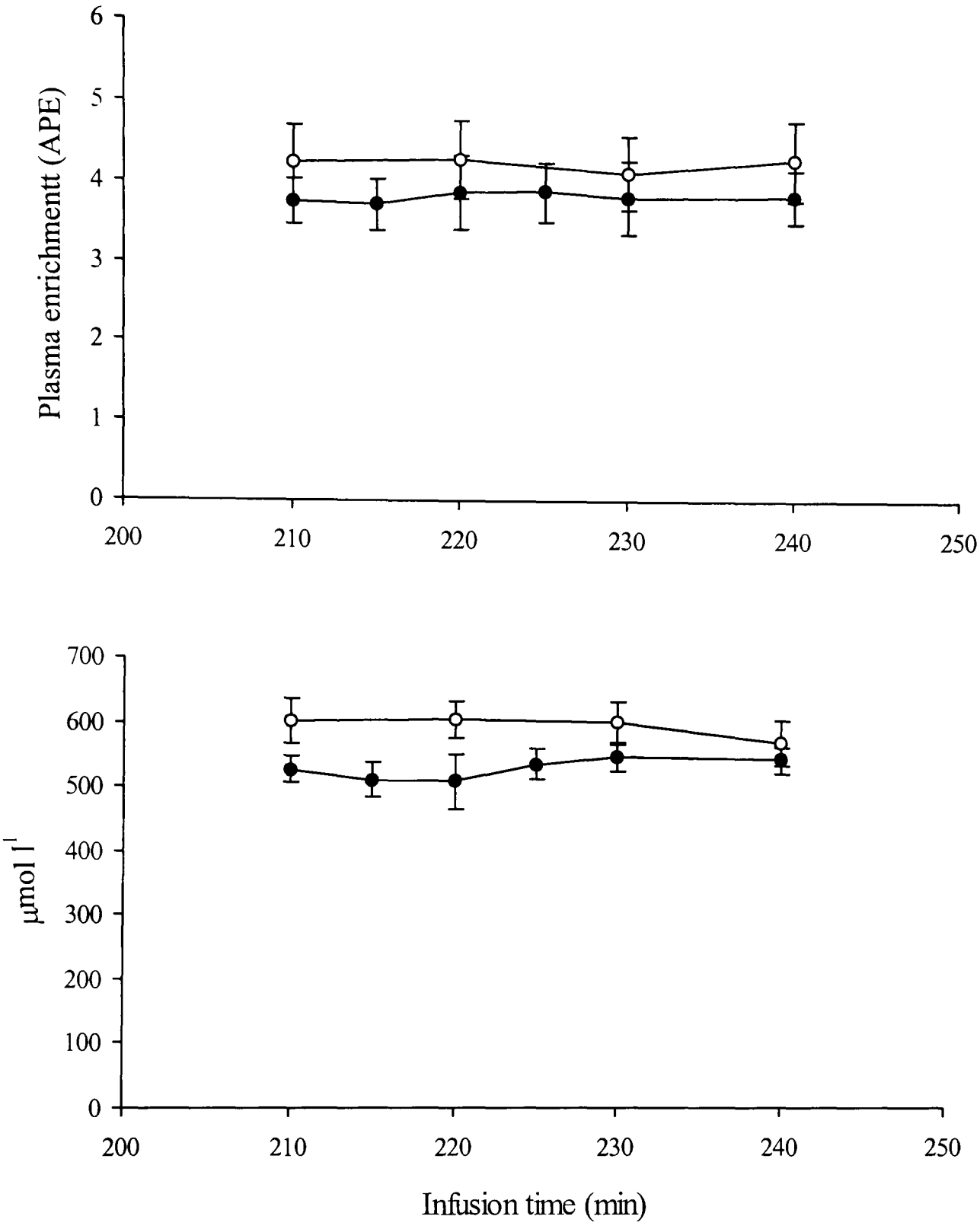


Figure 4.2. Plasma glutamine enrichment (APE) and concentration (µmol l^{-1}) for normal volunteers, young (○) and elderly (●). Values are means ± SE; *n*=6.

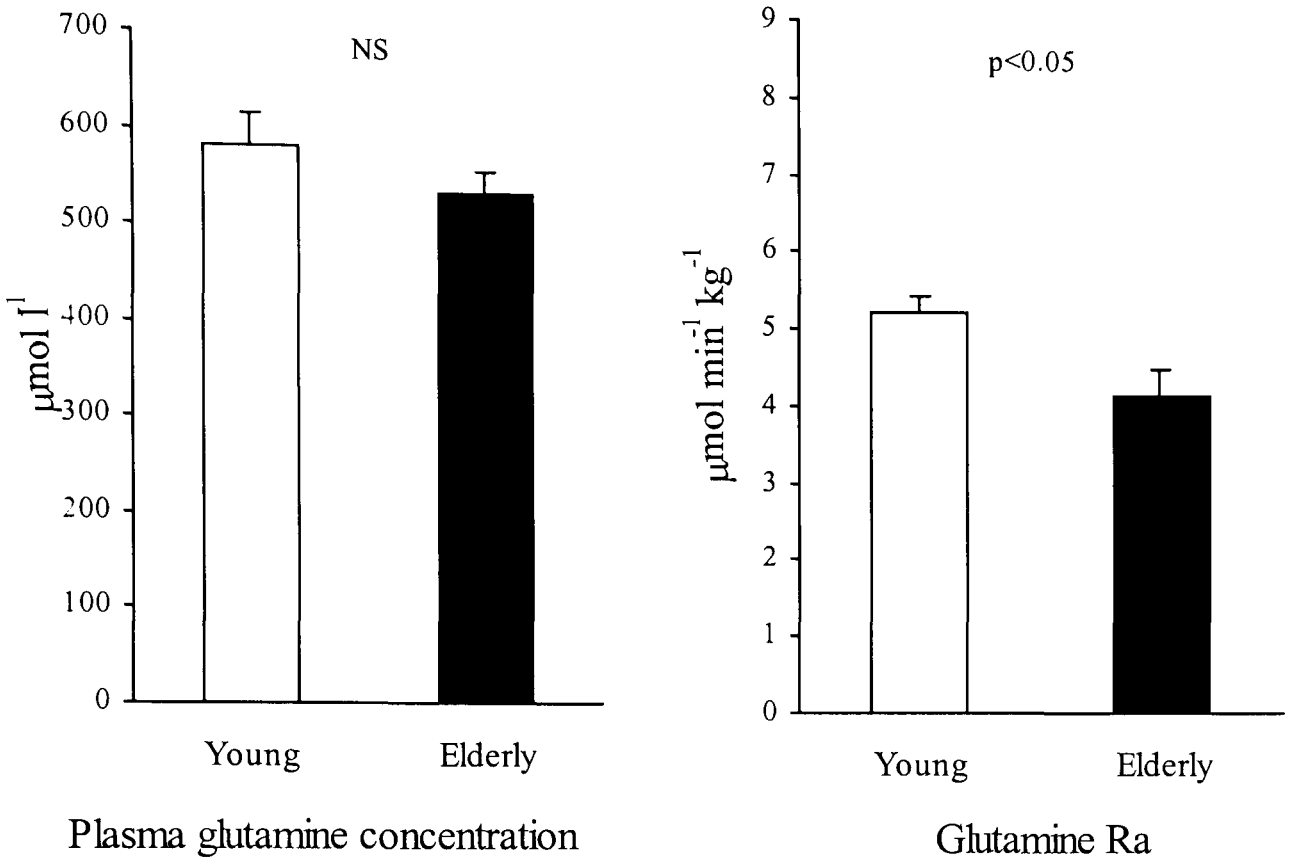


Figure 4.3. Plasma glutamine concentration, glutamine appearance rate and metabolic clearance rate for normal volunteers, young (□) and elderly (■). Values are means ± SE; *n*=6 in each group.

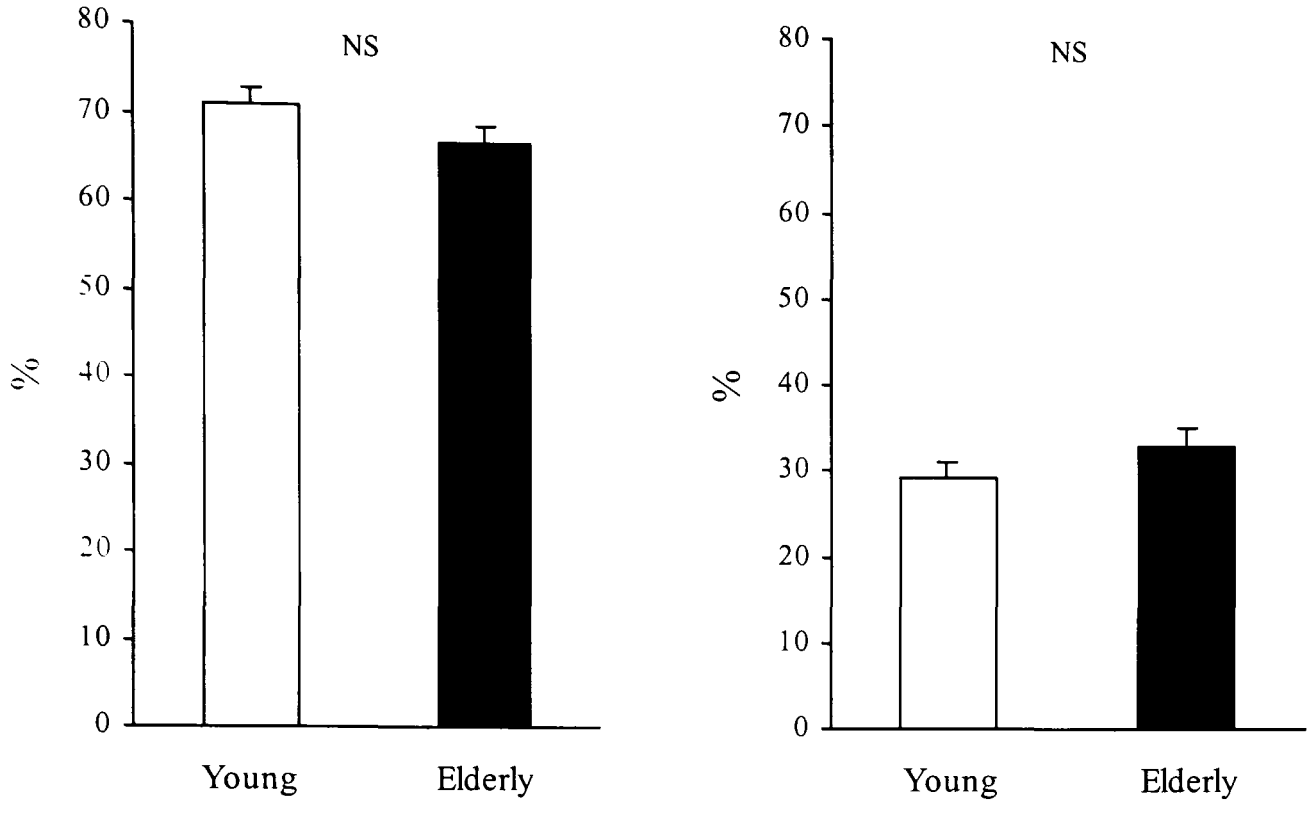
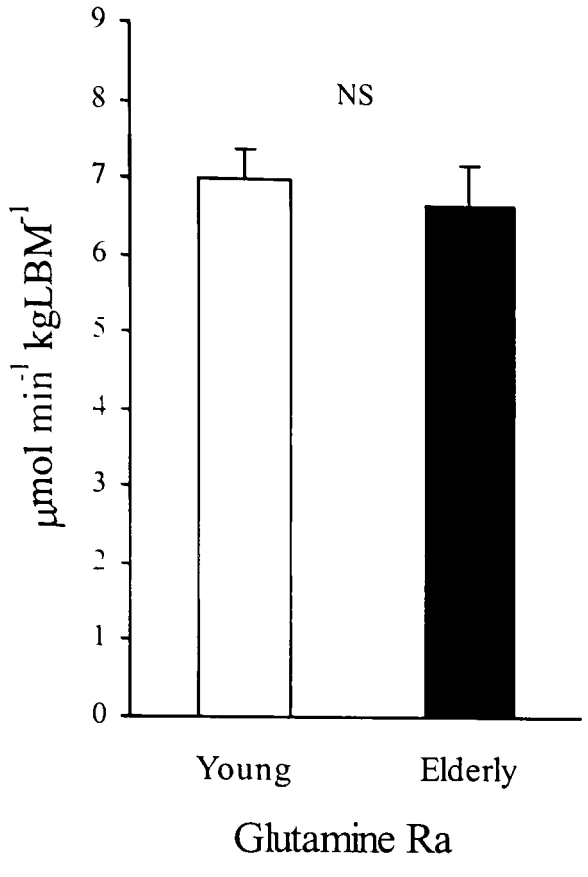


Figure 4.4. Percentage of glutamine appearance rate arising from de novo synthesis and proteolysis for normal volunteers, young (□) and elderly (■). Values are means ± SE; *n*=6 in each group.



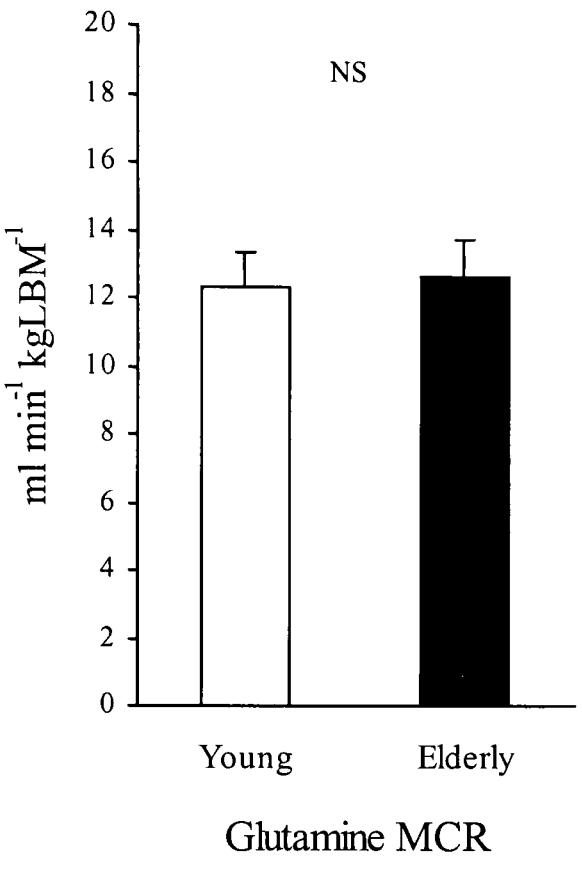


Figure 4.5. Glutamine appearance rate and metabolic clearance rate calculated per kg lean body mass for normal volunteers, young (□) and elderly (■). Values are means ± SE; *n*=6 in each group.

Discussion

In the present study the Ra_{gln} value in the young normal subjects was similar to previously published values for healthy adults in the same age range (e.g. Darmaun et al 1988, Matthews et al 1990, Brillon et al 1995). In contrast the Ra_{gln} value for the elderly group of normals was significantly lower than in the young group, and lower than previously published values for healthy adults using the same tracer (Table 4.4). However, the increase in body weight in the elderly group resulted from an increase in fat mass not a decrease in LBM. When the results were expressed per kg LBM there was no age associated decrease in Ra_{gln}, suggesting that the apparent difference in Ra_{gln} is related to age associated changes in body composition rather than altered glutamine metabolism. There is no previously published data reporting Ra_{gln} measurement in elderly normal subjects. In elderly patients (62 ± 3 years) with gastrointestinal cancer, the average Ra_{gln} following 2 hours of [5-^{15}N] glutamine infusion was 4.4 μmol min^{-1} kg^{-1} (Van Acker et al 1998).

Different tracers can give different values for plasma appearance rate depending on the metabolic fate of the portion of the molecule labelled. This has been previously shown for the non-essential amino acid alanine where the turnover rates measured using [^{15}N] labels are slower than those obtained using [^{13}C] labels (Yang et al 1984). This is because the flux of unlabelled carbon through the pyruvate pool is considerably greater than the flux of amino nitrogen through the transamination pool and hence the nitrogen label is more likely to be conserved after transamination and returned to an alanine molecule. The various parts of the glutamine molecule can enter a variety of different metabolic pathways at different rates. Glutamine is predominantly metabolised to glutamate with the loss of the amide nitrogen, glutamate is further metabolised to α-ketoglutarate with the loss of amino nitrogen and hydrogens (Kreider et al 1997). The carbons are lost when α-ketoglutarate enters the citric acid cycle.

Ra_{gln} has been measured in several studies in healthy adult subjects using a range of tracers (Table 4.4). Most of the early studies used nitrogen labelled tracers, in particular [2-^{15}N] glutamine, but recently investigators have begun to use carbon and hydrogen labelled tracers. Hankard and co-workers gave simultaneous infusions of

[1-^{13}C], [U-^{14}C], [3,4-^{3}H] glutamine to six healthy men (Hankard et al 1995). The Ra_{gln} values obtained (5.9, 6.2 and 6.6 μmol min^{-1} kg^{-1}, respectively) were not significantly different and were similar to the value previously reported by Darmaun et al 1986 using [2-^{15}N] glutamine (Hankard et al 1995). However, in a slightly larger study group (n=10), simultaneous infusions of [2-^{15}N], [U-^{14}C], and [3,4-^{3}H] glutamine gave significantly different Ra_{gln} values (Krieder et al 1997). The hydrogen labelled tracer gave Ra_{gln} estimates which were slightly, but significantly higher (7 %; $p< 0.05$) than those obtained with the carbon labelled tracer, which were in turn higher than the values obtained with the nitrogen labelled tracer (22 %; $p<0.01$). The authors suggest the difference in the hydrogen and carbon tracers could be due to recycling of glutamine carbon via a glucose-glutamine cycle (Perriello et al 1995) which would increase plasma glutamine specific activity and lower the apparent Ra_{gln}. The lower Ra_{gln} obtained with the nitrogen labelled tracer could also be partly due to recycling of the nitrogen label (Kreider et al 1997).

In 1986 Darmaun et al measured Ra_{gln} in post absorptive healthy adults using both [5-^{15}N] and [2-^{15}N] glutamine tracers. The Ra_{gln} value obtained with the amide tracer (4.7 μmol min^{-1} kg^{-1}) was ~80 % of the value given by the amino tracer (5.8 μmol min^{-1} kg^{-1}, $p<0.01$). The lower Ra_{gln} value obtained with the amide ^{15}N tracer could be explained if recycling of the label occurred, with reincorporation of ^{15}N ammonia released by glutaminase action back into glutamine via glutamine synthetase. This would increase the apparent [5-^{15}N] glutamine enrichment and lower the calculated Ra_{gln}. Darmaun considered this an unlikely explanation (as different enzymes add and remove the amide group) and instead proposed that the differences in Ra_{gln} values were due to loss of the amino label by reversible transamination. In rat tissues it has been shown that glutamine transaminates with a nunber of α-keto acids forming the corresponding amino acid and α-ketoglutaramate (Meister 1984). This would decrease the [2-^{15}N] glutamine enrichment resulting in a higher measure of Ra_{gln} (Darmaun et al 1986) however, there is no direct evidence for this at present.

Table 4.4. Glutamine production rates (μmol min^{-1} kg^{-1}) measured in healthy adults during i.v. glutamine tracer infusions.

Isotopic tracer						*No. of*	*Age*	*Source of information*
[2-^{15}N]	*[5-^{15}N]*	*[1-^{13}C]*	*[3,4-^{13}C]*	*[U-^{14}C]*	*[3,4-^{3}H]*	*subjects*		
5.8 ± 0.6	4.7 ± 0.5					5	23-26	Darmaun et al 1986
5.4 ± 0.5						6	23-30	Darmaun et al 1988
5.3 ± 0.2						5	25 ± 1	Matthews et al 1990
5.7 ± 0.4						5	24 ± 1	Matthews & Campbell 1992
4.9 ± 0.2						6	23-39	Matthews et al 1993
5.6 ± 0.3						6	33 ± 3	Darmaun et al 1994
	4.5 ± 0.3					5	28 ± 1	Gore & Jahoor 1994
5.6 ± 0.5						6	32 ± 2	Carbonnel et al 1995
		5.9 ± 0.4		6.2 ± 0.3	6.6 ± 0.4	6	24 ± 2	Hankard et al 1995
				5.8 ± 0.3		11	49 ± 2	Nurjhan et al 1995
			4.7 ± 0.2			8	29 ± 2	Hankard et al 1997
4.7 ± 0.4				5.7 ± 0.5	6.1 ± 0.5	10	28 ± 2	Kreider et al 1997
		5.1 ± 0.4				11	29 ± 3	Darmaun et al 1998

For a tracer to measure the overall rate of production of a substrate it should equilibrate fully with the entire whole body pool of the tracee. Darmaun et al 1986 showed that the infused [2-^{15}N] or [5-^{15}N] glutamine tracer equilibrates with a tracer miscible pool of only 207 μmol kg^{-1}, suggesting there is little mixing between plasma glutamine and the intracellular-muscle glutamine (5200 μmol kg^{-1}) and that the labelled glutamine does not trace intracellular metabolism. In our young normals we estimated a tracer miscible pool of 256 ± 15 μmol kg^{-1} close to the value reported by Darmaun. Darmaun concluded that the Ra_{gln} measured between 2-4 hours of tracer infusion represents inter-organ flux of glutamine rather than whole body glutamine production. However, inter-organ glutamine transport is considered to equate to glutamine metabolism, because the enzymes for its production are typically located in tissues (e.g. muscle) different from the tissues containing enzymes for glutamine degradation (e.g. gut) (Hankard et al 1995).

The model used to calculate Ra_{gln} assumes the whole body pool is a single, homogeneous instantly mixing compartment, and requires isotopic steady state. In a study in patients with gastrointestinal cancer in which [5-^{15}N] and [1-^{13}C] glutamine were infused for 11 hours both the plasma glutamine and muscle free glutamine enrichments increased, indicating isotopic steady state had not been achieved (Van Acker et al 1998). The authors suggest this is due to incomplete equilibration of the tracer with the large intramuscular free glutamine pool. This may lead to a considerable overestimation of whole body Ra_{gln} as there was a ~20 % decrease in the Ra_{gln} calculated after 11 hours of tracer infusion compared with the Ra_{gln} calculated after 2 hours. However, this data has not been reproduced by any other group. In the present study we sampled the steady state over the final 30 minutes of the tracer infusion, the plasma glutamine enrichments for the two groups of control subjects are shown in Figure 4.2. Repeated-measures ANOVA did not show a significant correlation with time, but this sampling period may have been too short to detect an increase in the plasma glutamine enrichment. In five of the normals we took earlier blood samples, the results shown in Figure 4.1 indicate an isotopic steady state between 3 and 4 hours. Using both [2-^{15}N] and [5-^{15}N] glutamine tracers with conventional GCMS sample analysis Darmaun and co-workers (1986) demonstrated

that the isotopic steady state in plasma was maintained between 2 and 4 hours of tracer infusion.

In the Van Acker study the glutamine isotopes were infused at a much lower rate than in the present study (0.12 vs. 2.5 mg h^{-1} kg^{-1}) and the ^{15}N and ^{13}C enrichments of glutamine were measured by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS). GC-C-IRMS is a much more sensitive technique than conventional GCMS and is capable of measuring much lower enrichments. Van Acker et al 1998 suggested that traditional GCMS methodology might not be precise enough to detect the small increases in plasma glutamine enrichment over this sampling period. It could be argued that Ra_{gln} was overestimated in the present study. However, in all our tracer infusion studies the tracer was infused for four hours and the isotopic "steady state" was sampled over the same period, thus the same errors will be incurred in each study.

As glutamine is a non-essential amino acid in the post-absorptive state Ra_{gln} is derived from protein breakdown (B_{gln}) and de novo synthesis (D_{gln}). If it is assumed that the release of an amino acid from proteolysis is proportional to its abundance in body protein, the rate of release of a non-essential amino acid from proteolysis can be estimated from the appearance rate of an essential amino acid (e.g. leucine) when tracers are infused simultaneously into the same subjects (Robert et al 1982). Although body protein is known to contain 13.9 g of glutamine plus glutamate per 100 g, the exact glutamine content is not known. During in vitro acid hydrolysis of protein the glutamine residues are degraded to glutamate, therefore most protein composition tables only provide the total glutamine plus glutamate levels (Hankard et al 1995). This total glutamine plus glutamate value has been used to calculate the proportion of Ra_{gln} derived from protein synthesis, but this leads to underestimation of the calculated de novo glutamine synthesis rate (Darmaun et al 1988, Nurjhan et al 1995). Researchers have estimated that glutamine contributes half the total glutamine plus glutamate value i.e. 7 g per 100 g protein (Matthews and Campbell 1992, Hankard et al 1997, Darmaun et al 1998). Regardless of the glutamine content chosen it must be emphasised that the calculated values of B_{gln} or D_{gln} are only estimates, but since, the true glutamine content of whole body protein is likely to be constant the direction of changes in B_{gln} or D_{gln} should be valid (Hankard et al 1995). The proportions of the

Ra_{gln} estimated as arising from de novo glutamine synthesis (71 %) and from proteolysis (29 %) in the young normals were similar to previously reported values for young healthy volunteers of a similar age (68 % and 32-36 %, respectively) (Hankard et al 1997, Darmaun et al 1998). D_{gln} (67 %) and B_{gln} (33 %) for the elderly normals were also close to these values. This probably reflects the fact that muscle tissue is the main site of glutamine production in the post-absorptive state (Van Acker et al 1998) and the measured lean body mass was similar in the two groups of normals in our study.

There was a wide range of GH levels in both the young (0.63-16.99 mU l^{-1}) and elderly (0.68–9.53 mU l^{-1}) normals. The values we have quoted are for samples taken during the metabolic tracer study and not an average for 24 hour profiles. Because of the pulsatile nature of GH secretion it is difficult to report a normal range. The average GH levels in our controls are similar to the mean GH value (4.0 ± 0.98 mU l^{-1}) reported following a 24 hour profile in six elderly fasting normals (64 ± 4 years) (Ross et al 1991a). IGF-I levels were lower in the elderly normal; decreasing IGF-I levels with age is well known and may be linked to reduced GH secretion. The IGF-I levels for the elderly normals were all within the normal range for >60 year olds (3.7-32.9 nmol l^{-1}). Insulin and C-peptide levels tended to be higher in the elderly normals, although this increase was not significant. However, the plasma glucose levels were significantly increased. This suggests increased insulin resistance probably due to their increased fat mass. The young normals were recruited from members of the department and so were within their normal working environment and also had previous experience of metabolic tracer studies. The elderly normals may have been stressed by their visit to the hospital and by the study protocol, this may explain the higher cortisol levels found in this group. Although the cortisol levels were higher in the elderly normals they were still within the normal range (120-500 nmol l^{-1}, 9:00 a.m. sample).

It is well recognised that LBM decreases with age, so it was surprising to find similar LBM measurements for the two groups of normals. Body composition measurements vary in sophistication from reference methods (e.g. measurement of total body potassium), which are only available in research centres, to simple bedside measurements (e.g. bioelectric impedance) (Manning and Shenkin 1995, Enzi et al

1997). Bioelectric impedance is the most accessible method to measure body composition and was therefore used in the controls in the present study. This technique uses prediction equations which take into account body weight, height and gender to convert the impedance measurement into an estimate of total water volume, from which LBM is estimated (assuming a constant hydration of lean tissue). However, a large variation in the hydration of lean tissue has been reported for healthy subjects (69-76 %; Streat et al 1985) which increases with age (64-80 %: Sergi et al 1993). A recently published study has questioned the accuracy of bioelectric impedance analysis in the elderly (due to the variability in lean tissue hydration) and suggests that further validaion studies are necessary (Bussolotto et al 1999). In the present study the elderly normals tended to be heavier than the young normals although the difference was not statistically significant. If we had been able to match two groups of normal subjects for weight it is likely that LBM would have been lower in the elderly group.

As LBM is normally lower in older subjects it is clearly important to match for LBM when comparing glutamine metabolism in critically ill patients with normal subjects. Many of the techniques used to estimate body composition (measurement of total body potassium, in vivo neutron activation analysis, and dual energy x-ray absorptiometry) require the subjects to be moved to whole body scanners or counters. This makes them impractical for use in critically ill patients who may be attached to ventilators and/or hemodialysis machines (Manning and Shenkin 1995). In addition, there is some doubt about the practicality and validity of bioelectrical impedance measurements of body composition in critically ill patients as the results may be affected by the hydration status of the patient (Manning and Shenkin 1995, Jacobs 1996). Fluid retention (overestimate) or dehydration (underestimate) will affect the estimation of LBM. As most methods of measuring body composition appear to have severe limitations in the critically ill these results suggest it is important to match the controls for age and BMI when measuring glutamine metabolism in ICU patients.

Chapter 5

GLUTAMINE METABOLISM IN THE CRITICALLY ILL

Introduction

Glutamine is the most abundant amino acid in both plasma and the free intracellular amino acid pool in skeletal muscle (Bergstöm et al 1974). Because most tissues have the ability to synthesise glutamine it is defined as a non-essential amino acid. However, free glutamine concentrations are extremely labile and marked decreases have been reported in a variety of catabolic states (Askanazi et al 1980, Vinnars et al 1975). This suggests that during serious illness a deficiency in glutamine availability may develop and has led to the idea that glutamine is a conditionally essential amino acid (Lacey and Wilmore 1990).

The measurement of whole body plasma glutamine metabolism using glutamine labelled with ^{15}N has been developed, allowing experimental and clinical investigations of glutamine metabolism. Healthy subjects and the effects of various catabolic hormones on glutamine metabolism have been studied (Darmaun et al 1986, Darmaun et al 1988, Brillon et al 1995, Matthews et al 1990, Gore and Jahoor 1994). However, despite the current clinical interest in the benefits of glutamine supplementation there have been very few tracer studies investigating glutamine metabolism in patient groups, particularly critically ill patients. To our knowledge there have been no published reports of studies using stable isotope tracer techniques to measure glutamine metabolism in critically ill patients soon after the onset of illness in a general ICU.

Aim

The aim of the present study was to use L-[2-^{15}N] glutamine to investigate whole body glutamine metabolism in a group of critically ill patients and a group of matched healthy controls.

Subjects

Seven severely ill patients (age range 32-76 years) in the ICU of St Thomas' Hospital were studied, details of their clinical and metabolic characteristics are given in Table 5.1. Five of the patients had undergone emergency abdominal surgery within 24 hours

of the start of the study. A continuous insulin infusion (Actrapid; Novo Nordisk, Copenhagen, Denmark) was provided if necessary, as part of the clinical care, to maintain plasma glucose concentration at or below 7 mmol l^{-1}. Seven of the normal subjects from Chapter 4, matched for gender, age, weight and BMI, were selected as healthy controls for comparison; their physical characteristics are also shown in Table 5.1. The study protocol is described in Chapter 2.

Results

Table 5.1 shows the details of the seven critically ill patients studied. The severity of the illness is indicated by the TISS and APACHE II scores (Cullen et al 1974, Knaus et al 1985). These indices identified the patients as being severely ill and dependent on cardiorespiratory and nutritional support. None of the patients were acidaemic (pH 7.44 ± 0.02). Only two of the patients were given exogenous insulin infusions during the study, patients 1 and 6 received 2 and 1 U hr^{-1} respectively.

Metabolite and hormone profiles are shown in Table 5.2. There was no difference in the plasma glucose between the two groups. Although plasma insulin levels tended to be higher in the critically ill patients, this did not reach statistical significance ($p=0.062$, range 8.5-29 mU l^{-1}). C-peptide levels were significantly increased in the patients ($p<0.01$). GH levels were similar in both groups. Total IGF-I and IGFBP-3 were significantly lower in the patient group ($p<0.05$ and $p<0.001$). Although the mean IGFBP-1 levels were more than doubled in the patients this increase was not statisically significant ($p=0.14$, range 22-238 ng ml^{-1}). Cortisol levels were higher in the patients, but again this increase did not reach statistical significance ($p=0.069$, range 242-2393 nmol l^{-1}). Glucagon levels were significantly higher in the patient group ($p<0.001$). Thyroid hormone levels were significantly lower ($p<0.05$) in the patients.

Table 5.1. Characteristics of critically ill patients and matched healthy controls

Subject No.	*Gender*	*Age (yrs)*	*Weight (kg)*	*Height (cm)*	*BMI (kg m^{-2})*	*APACHE II*	*TISS*	*Plasma Albumin (g l^{-1})*	*Plasma CRP (mg l^{-1})*	*Main Diagnosis*
ICU patients										
1	F	68	64.0	171	22.2	15	34	19	157	Bowel resection
2	M	76	73.6	182	22.2	23	49	27	68	GI bleed/laparotomy
3	M	72	82.0	187	23.4	15	37	23	161	GI obstruction
4	F	65	75.0	170	26.0	9	50	34	82	Bowel resection
5	F	75	85.0	168	30.1	19	38	25	329	Bowel obstruction
6	M	75	103.7	183	31.0	24	47	19	248	AAA
7	F	32	58.5	170	20.2	18	62	17	145	Bowel resection
mean ± SE	**3M:4F**	**66 ± 6**	**77.4 ± 5.6**	**176 ± 3**	**25.0 ± 1.6**	**18 ± 2**	**45 ± 4**	**23 ± 2**	**170 ± 35**	
Matched controls										
1	F	62	62.8	170	21.7					
2	M	77	85.5	174	28.2					
3	M	65	86.6	185	25.3					
4	F	67	76.2	156	31.3					
5	F	65	78.9	158	31.8					
6	M	70	91.5	174	30.2					
7	F	29	62.0	171	21.2					
mean ± SE	**3M:4F**	**62 ± 6**	**77.6 ± 4.4**	**170 ± 4**	**27.1 ± 1.7**		*Reference ranges:*	35 - 46	< 7	

BMI, body mass index; APACHE II, acute physiology and chronic health evaluation; TISS, therapeutic intervention scoring system; CRP, C-reactive protein; GI, gastrointestinal; AAA, aortic abdominal aneurysm.

Table 5.2. Hormone and metabolite concentrations of critically ill patients and matched healthy controls. Values are means ± SE, *n*=7.

	Units	*Healthy Controls*	*Critically Ill Patients*	*p*
Glucose	mmol l^{-1}	5.7 ± 0.2	6.0 ± 0.5	NS
Insulin	mU l^{-1}	10.9 ± 2.5	17.5 ± 2.6	NS
C-peptide	nmol l^{-1}	0.44 ± 0.11	1.63 ± 0.36	< 0.01
GH	mU l^{-1}	4.42 ± 1.03	3.75 ± 1.10	NS
IGF-I	nmol l^{-1}	17.0 ± 1.5	12.4 ± 0.7	< 0.05
IGFBP-1	ng ml^{-1}	38 ± 5	103 ± 36	NS
IGFBP-3	ng ml^{-1}	2218 ± 282	1219 ± 142	< 0.001
Cortisol	nmol l^{-1}	318 ± 41	784 ± 288	NS
Glucagon	pg ml^{-1}	61 ± 6	302 ± 52	< 0.001
Free Thyroxine	pmol l^{-1}	14.5 ± 0.6	11.4 ± 1.2	< 0.05
Free Tri-iodothyronine	pmol l^{-1}	4.6 ± 0.2	3.0 ± 0.5	< 0.05

The plasma amino acid profiles are listed in Table 5.3. The plasma glutamine concentration was significantly lower in the critically ill patients ($p<0.001$). Plasma threonine ($p<0.01$), serine ($p<0.001$), glycine ($p<0.01$) alanine ($p<0.001$), leucine ($p<0.05$), lysine, ($p<0.01$), histidine ($p<0.01$) and arginine ($p<0.01$) concentrations were significantly lower in the patients, whereas phenylalanine ($p<0.01$) and aspartate ($p<0.01$) levels were significantly higher. There was no significant difference in BCAA, but total amino acids were significantly lower ($p<0.001$) in the patients.

Figure 5.1 shows the plasma glutamine enrichments and concentrations for the critically ill patients and their matched controls during the final 30 minutes of the tracer infusion. The glutamine turnover data for the patients and their matched controls are summarised in Figure 5.2. MCR_{gln} was significantly higher in the critically ill patients compared with the matched controls (14.92 ± 0.68 vs. 7.85 ± 0.47 ml min^{-1} kg^{-1}; $p<0.001$). There was no difference in whole body Ra_{gln} (or Rd_{gln}) between the two groups (4.89 ± 0.31 vs. 4.24 ± 0.33 μmol min^{-1} kg^{-1}). B_{gln} was significantly higher in the patients compared to the matched controls (2.06 ± 0.13 vs. 1.32 ± 0.08 μmol min^{-1} kg^{-1}; $p<0.001$). When expressed as a percentage of Ra_{gln} there was a significant increase in the proportion of Ra_{gln} arising from protein breakdown in the patients (43 ± 3 vs. 32 ± 2 %; $p<0.05$) (Figure 5.3). D_{gln} was similar in both groups (2.83 ± 0.28 vs. 2.92 ± 0.26 μmol min^{-1} kg^{-1}), but when expressed as a percentage of Ra_{gln} there was a significant decrease in the proportion of Ra_{gln} arising from de novo synthesis in the critically ill patients (57 ± 3 vs. 68 ± 2 %; $p<0.05$) (Figure 5.3).

Table 5.3. Plasma amino acid profiles of critically ill patients and matched healthy controls. Values are means ± SE, *n=7*.

Amino acid ($\mu mol\ l^{-1}$)	*Matched Controls*	*Critically Ill Patients*	*p*
Aspartate	5 ± 0.5	17 ± 6	< 0.01
Threonine	122 ± 18	48 ± 6	< 0.01
Serine	98 ± 8	50 ± 6	< 0.001
Glutamate	59 ± 9	34 ± 8	NS
Glutamine	540 ± 53	329 ± 21	< 0.001
Glycine	204 ± 18	129 ± 13	< 0.01
Alanine	276 ± 23	139 ± 15	< 0.001
Valine	205 ± 18	151 ± 20	NS
Cystine	65 ± 5	56 ± 13	NS
Methionine	30 ± 9	12 ± 2	NS
Isoleucine	34 ± 5	33 ± 13	NS
Leucine	129 ± 9	95 ± 12	< 0.05
Tyrosine	39 ± 7	40 ± 6	NS
Phenylalanine	42 ± 4	72 ± 7	< 0.01
Ornithine	44 ± 4	34 ± 3	NS
Lysine	159 ± 16	88 ± 6	< 0.01
Histidine	63 ± 3	39 ± 4	< 0.01
BCAA	368 ± 32	274 ± 37	NS
Total	2214 ± 100	1382 ± 95	< 0.001

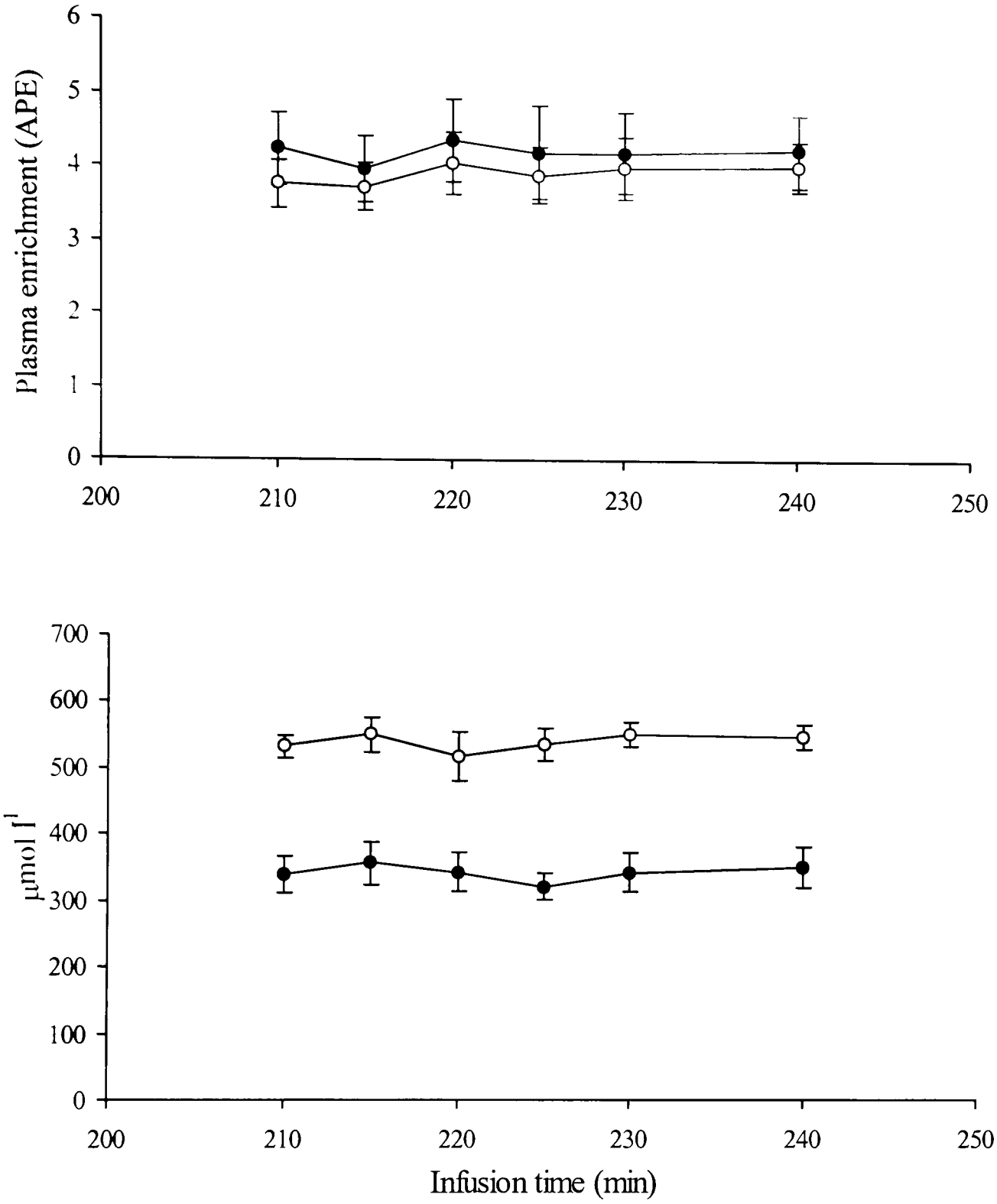
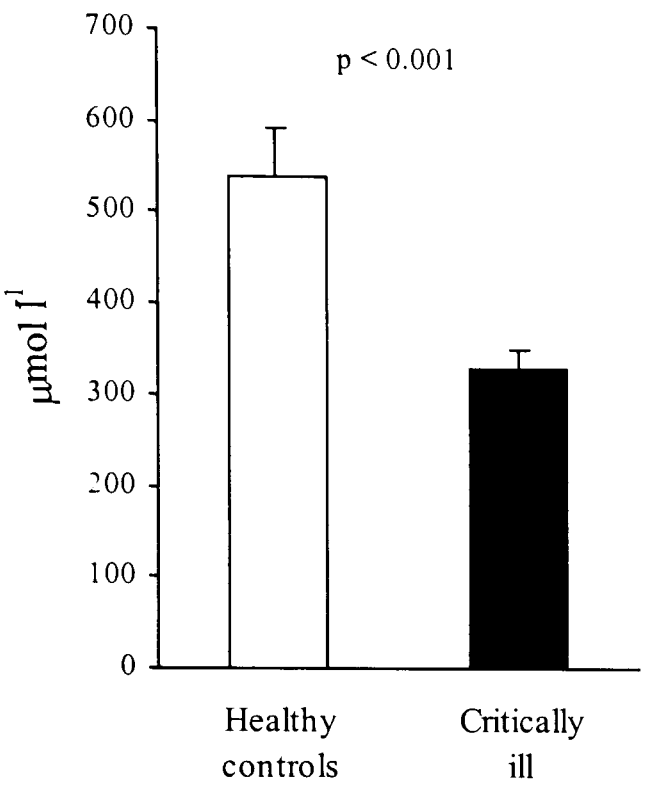
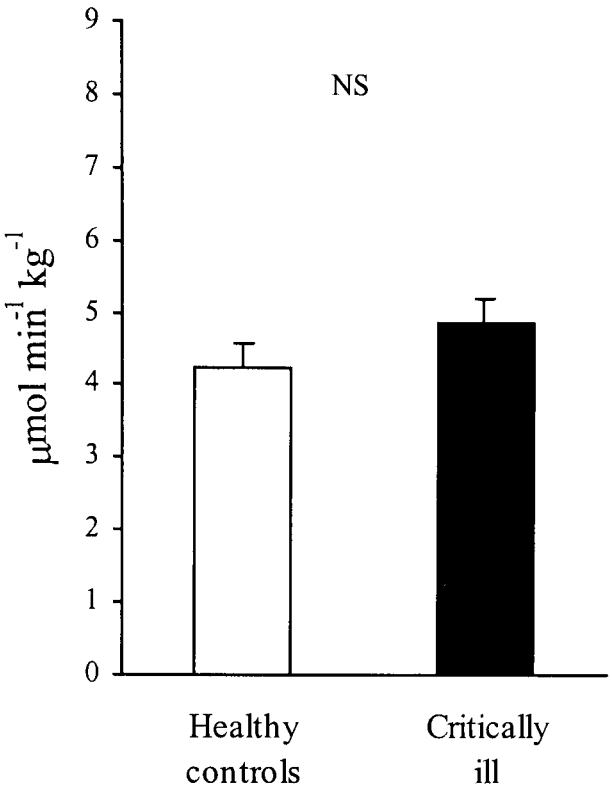


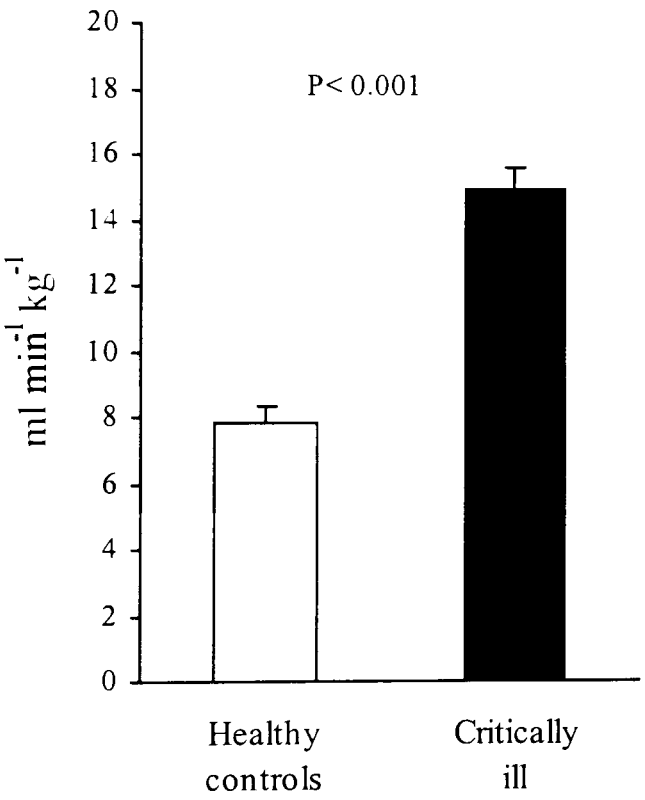
Figure 5.1. Plasma glutamine enrichment (APE) and concentration (μmol l^{-1}) for critically ill patients (●) and matched controls (○). Values are means ± SE; *n*=7.



Plasma glutamine concentration



Glutamine Ra



Glutamine MCR

Figure 5.2. Plasma glutamine concentration, glutamine appearance rate and metabolic clearance rate for critically ill patients (■) and matched healthy controls (□). Values are means ± SE; *n*=7 in each group.

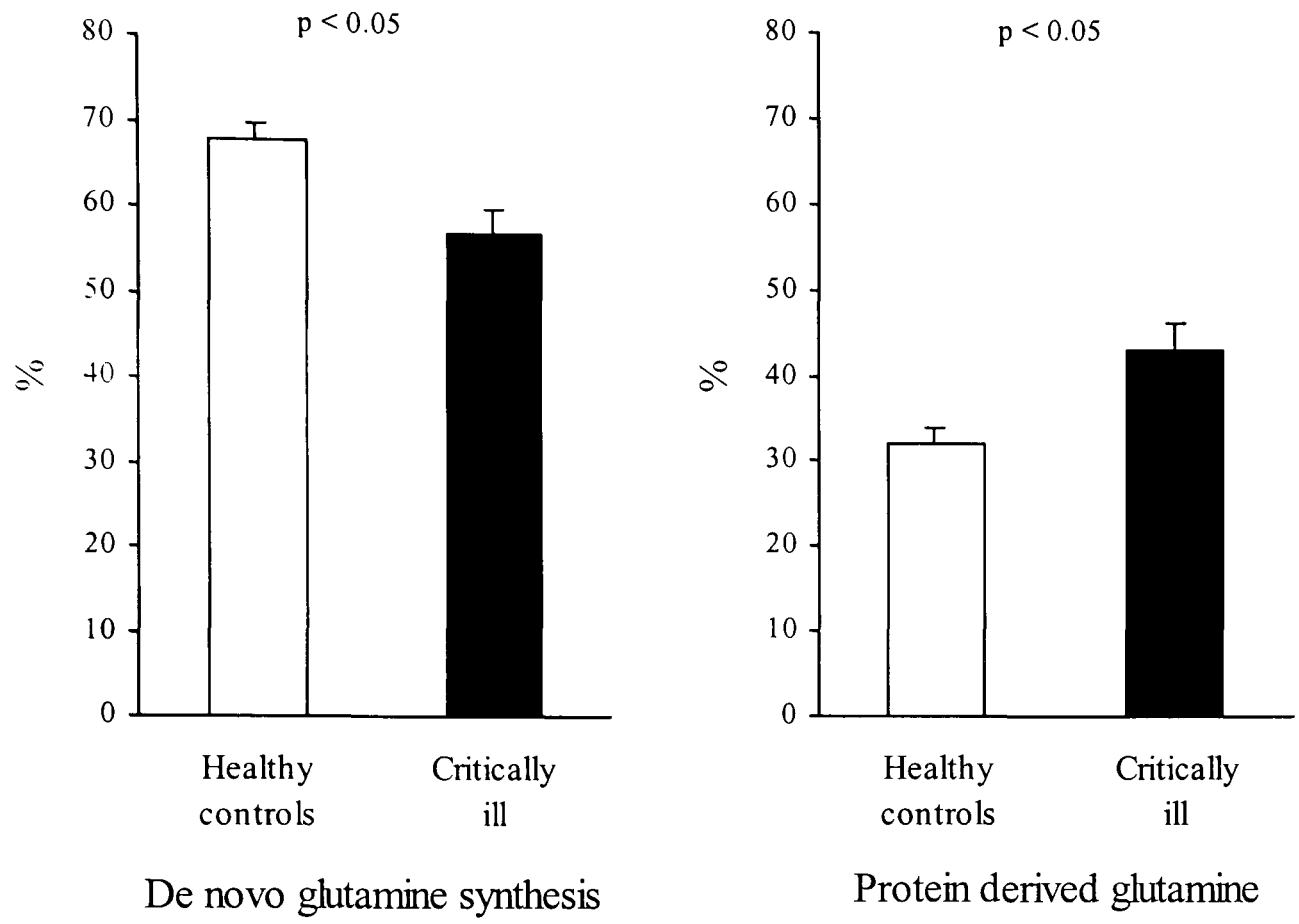


Figure 5.3. Percentage of glutamine appearance rate arising from de novo synthesis and proteolysis for critically ill patients (■) and matched healthy controls (□). Values are means ± SE; *n*=7 in each group.

Discussion

There is currently intense clinical interest in glutamine metabolism in critical illness. Previous studies have reported whole body glutamine turnover measurements in burns patients (Gore and Jahoor 1994), enterectomized patients (Darmaun et al 1991a), patients with nonneoplastic gastrointestinal disease (Carbonnel et al 1998), patients with active coeliac disease (Messing et al 1998) and in type 1 diabetics (Darmaun et al 1991b). There have been no previous studies using isotopic tracers to investigate glutamine metabolism in acute critical illness. In this study we have shown that despite a marked decrease in plasma glutamine concentration, whole body Ra_{gln} was unchanged in critically ill patients. Whole body Ra_{gln} reflects inter-organ glutamine transport rates through plasma (Darmaun et al 1986). However, the percentage of Ra_{gln} arising from protein breakdown was increased and the percentage from de novo glutamine synthesis was decreased in this patient group. The observed decrease in plasma glutamine concentration suggests that this increase in protein derived glutamine was insufficient to meet the increased demand for glutamine.

MCR_{gln} was increased in the critically ill patients suggesting that this may be the primary mechanism for the fall in glutamine concentration. MCR_{gln} reflects the rate at which glutamine is cleared from the plasma pool. Since amino acids are removed from blood by a transporter we would expect this removal to exhibit Michaelis-Menten kinetics (i.e. non-linear kinetics). There will thus be an inverse relationship between clearance and concentration. Thus if Ra_{gln} decreases concentration would be expected to fall and clearance to rise. However glutamine Ra_{gln} and Rd_{gln} were unchanged in the critically ill patients despite a fall in glutamine concentration. The increase in MCR_{gln} in the patients is therefore likely to be due to a change in the transport process e.g. an increase in efficiency.

There were marked differences in the levels of plasma amino acids between the patients and their matched controls. The decreased glutamine concentrations seen in the patients in the present study were similar to previously published values for critically ill patients (365 ± 126 μmol l^{-1}; Gamrin et al 1996). It has been suggested that the decrease in glutamine levels may indicate an inability of glutamine synthetic mechanisms to meet the increased metabolic demand of critical illness. Thus glutamine may behave as a "conditionally essential" amino acid. The concentrations of

the essential amino acids, leucine, lysine, threonine and histidine were also lower in the patients. This has been reported in previous studies of critically ill patients (Gamrin et al 1996, Roth et al 1982). The decrease in plasma amino acid concentrations may be due to increased splanchnic amino acid uptake as a result of the elevated levels of stress hormones (Wernerman et al 1985). In contrast there was an increase in the plasma phenylalanine concentration. This response has also been observed in previous studies of critically ill patients and it has been shown that with continuing illness phenylalanine concentrations continue to rise (Gamrin et al 1996, Roth et al 1982). As liver is the major organ of phenylalanine hydroxylation and oxidation, the increasing phenylalanine levels may be an indicator of liver damage in these patients or may simply reflect the increased phenylalanine release from muscle exceeding its utilization.

It has been shown that there is an integtrated metabolic response to critical illness increased concentrations of the "catabolic" hormones (catecholamines, glucocorticoids and glucagon) and with elevated levels of cytokines and inflammatory mediators (Van den Berghe et al 1998). In the patients in the present study free thyroid hormone concentrations were decreased. Low serum T_3 and T_4 levels are typical of the thyroid status in critically ill patients which has been called "sick euthyroid syndrome" (Docter et al 1993). Glucagon levels were increased in the patients and although cortisol levels also tended to be higher, the increase did not reach statistical significance. Ross et al 1991(b) reported elevated mean 24 hour cortisol levels with loss of the normal circadian rhythm in critically ill patients. C-peptide levels were increased in the patients indicating increased endogenous insulin secretion, although the increase in insulin levels was not significant.

Critical illness is associated with changes in the GH/IGF-I axis which include low circulating IGF-I, IGFBP-3 and ALS levels with normal or high IGFBP-1 levels (Ross et al 1991a, Ross et al 1991b). In our study the IGF-I and IGFBP-3 levels were lower in the critically ill patients, and IGFBP-1 levels tended to be higher although this increase was not significant. These changes are characteristic of the "acquired GH resistance" of critically ill patients and are thought to reduce the indirect protein anabolic effects of GH which are mediated by IGF-I (Ross et al 1991a, Ross et al 1991b). Unfortunately, we were unable to take sufficient samples to establish GH

profiles, and the GH values reported are from samples taken during the metabolic tracer study. Because of the pulsatile nature of GH secretion, these samples do not provide very useful information regarding GH status. Despite the "acquired GH resistance" of ICU patients the GH levels in our patients were similar to the matched controls. However, Ross and co-workers compared the 24 hour GH profiles in six critically ill patients with matched healthy controls and showed that although the baseline GH levels were higher in the critically ill (3.1 ± 0.8 vs. 0.8 ± 0.3 mU l^{-1}) the mean GH levels were similar (4.5 ± 0.8 vs. 4.0 ± 1.0 mU l^{-1}) in both groups (Ross et al 1991a).

Animal studies suggest that glucocorticoids are important mediators of the altered glutamine metabolism that occurs during stress. Seven days of dexamethasone treatment in dogs (0.44 mg kg^{-1} day^{-1}) increased glutamine release from hindquarter skeletal muscle resulting in a decreased intracellular free glutamine concentration (Muhlbacher et al 1984). Despite the increased glutamine release, plasma levels of glutamine also fell (~26 %) suggesting increased uptake by other tissues. Dexamethasone treatment has also been shown to stimulate the uptake and utilisation of glutamine by the kidneys in the dog (Souba et al 1985) and the intestine in the dog (Souba et al 1985) and in the rat (Ardawi et al 1988).

Previous studies have investigated the effects of catabolic hormone infusions on glutamine metabolism in healthy volunteers. Using [2-^{15}N] glutamine as a tracer, Darmaun and colleagues infused hydrocortisone (140 μg h^{-1} kg^{-1} for 64 hours) into healthy volunteers to raise plasma cortisol levels into the range normally seen in critically ill patients (Darmaun et al 1988). The modest hypercortisolemia (35 μg dl^{-1}) increased whole body Ra_{gln} by 40 %. The increase in Ra_{gln} was primarily due to a 55 % increase in de novo glutamine synthesis, and resulted in a significant increase in the plasma glutamine concentration. This cortisol mediated increase in Ra_{gln} has been shown to be dose dependent (Brillon et al 1995). In this experiment, 17 hour infusions of hydrocortisone at 80 or 200 μg h^{-1} kg^{-1}, increased plasma cortisol levels to 34 and 65 μg dl^{-1} and increased Ra_{gln} by ~23 and ~40 % respectively. The majority (~80 %) of this increase was accounted for by increased de novo glutamine synthesis. A short term increase in Ra_{gln} could occur by contraction of the muscle intracellular free

glutamine pool, and the tracer methodology used to estimate de novo glutamine synthesis does not distinguish between increased glutamine release from the intracellular muscle free glutamine pool or increased glutamine synthesis and release by muscle (Brillion et al 1995).

An infusion of epinephrine (2 μg min^{-1} for 8.5 hours) caused only a modest increase in whole body Ra_{gln} (7 %) in healthy adults (Matthews et al 1990). This infusion rate resulted in plasma epinephrine levels of ~750 pg ml^{-1} which are typical of the levels seen in trauma and sepsis. In contrast, a triple hormone infusion of epinephrine, cortisol and glucagon increased whole body Ra_{gln} and MCR_{gln}, and decreased plasma glutamine concentration (Gore and Jahoor 1994). The plasma cortisol (2538 ± 248 nmol l^{-1}) and glucagon (498 ± 102 ng l^{-1}) levels achieved in these volunteers were much higher than the levels seen in our critically ill patients. These studies suggest that these counter regulatory hormones may regulate the rate of glutamine metabolism, possibly through effects on glutamine transporters (McGivan and Pastor-Anglada 1994), however they cannot fully mimic the complex changes occurring in critically ill patients.

Although there are no comparable studies in critically ill patients, whole body Ra_{gln} has been measured in patients following burn injury using a similar stable isotope technique (Gore and Jahoor 1994). Ra_{gln} was higher in burn patients (7.2 ± 0.6 μmol min^{-1} kg^{-1}), in comparison with the values we obtained for critically ill patients (4.9 ± 0.3 μmol min^{-1} kg^{-1}) in the current studies. Although the decreases in plasma glutamine concentration were similar in the two studies, a significant increase (60 %) in Ra_{gln} was reported in the burns patients compared to a control group, in contrast to the unchanged value in the present study. These changes were associated with a marked increase in glutamine MCR_{gln} in the burns patients (~200 %) compared with control subjects, in contrast to the 92 % increase recorded in our patient group. However, these measurements were made 2 weeks after the burn injury whereas the patients in the present study were studied within 24 hours of admission to the ICU, and often within hours of emergency surgery. Although the plasma glucagon levels in the burns patients were similar to our ICU patients, the cortisol levels were much higher (1848 ± 579 nmol l^{-1}). The burns patients had been receiving enteral nutrition which was withdrawn 8 hours prior to the study. An increase in whole body Ra_{gln} (28 %) has

also been shown in chronically malnourished, nonstressed adults with noncancer gastric diseases (BMI ~16 kg m^{-2}) (Carbonnel et al 1995).

Phenylbutyrate treatment (0.36 g kg^{-1} day^{-1}) has been used to create a "stress-free model" of glutamine depletion in healthy adults by "trapping" glutamine (Darmaun et al 1998). The proposed mechanism involves the conversion of phenylbutyrate to phenylacetate by β-oxidation which in turn reacts with glutamine in the liver and kidneys to form phenylacetylglutamine, which is excreted by the kidneys. The acute glutamine depletion (~26 % fall in plasma glutamine levels) induced by 1 day of phenylbutyrate treatment, did not affect whole body Ra_{gln} but increased MCR_{gln} by ~34 %. There were no compensatory increases in the rates of de novo glutamine synthesis and/or release from protein breakdown, (Darmaun et al 1998). This suggests hormone changes and increased glutamine requirements play an important role in mediating the increased Ra_{gln} previously reported for burns patients (Gore and Jahoor 1994).

Critically ill patients are a difficult group to study in large numbers for two main reasons; 1) the difficulties of conducting studies in severely ill patients and 2) the heterogeneity of patients treated in an intensive care unit in terms of severity of illness, nutritional status, and timing of studies, make it difficult to classify and compare individuals. Consequently much of the research investigating the effects of glutamine supplementation has used metabolically healthy patients with stable circulation and minimal preoperative blood loss undergoing planned operative intervention (standardised surgical procedures: e.g. elective cholecystectomy).

In this study the patients recruited in the ICU were those in whom the clinical decision had been made to use TPN. Although these patients were a heterogeneous group, in contrast to previous studies of glutamine metabolism, they represent the group in whom glutamine supplementation may have potential benefit. The study demonstrates that the increased glutamine clearance associated with a normal Ra_{gln} resulted in a decrease in glutamine concentration, suggesting that the increase in protein breakdown was insufficient to meet the demand for glutamine in these catabolic patients.

Chapter 6

EFFFECT OF NUTRITIONAL SUPPORT ON GLUTAMINE METABOLISM IN CRITICAL ILLNESS

Introduction

During critical illness amino acids are mobilised from peripheral tissue, such as muscle, and are used by organs in the splanchnic area for gluconeogenesis, oxidation, ureagenesis, protein synthesis and also as substrates for the immune system and wound healing (Gamrin at al 1996). This is thought to lead to the wasting of lean body mass characteristic of critical illness which often persists despite nutritional support. Because maintenance of body protein stores may have an impact on morbidity and mortality several strategies are currently being investigated to prevent the loss of lean tissue, including the use of specialised nutrition.

At present glutamine is not routinely added to TPN, but recent clinical trials suggest that glutamine supplementation improves both nitrogen balance and gut mucosal integrity and decreases the number of infections, length of hospital stay, and 6 month mortality in critically ill patients (Van der Hulst et al 1993, Ziegler et al 1992, Griffiths et al 1997). Despite the current clinical interest in the potential benefits of glutamine supplementation there have been no tracer studies investigating the effects of glutamine supplementation on glutamine metabolism in critically ill patients.

Aim

The aim of the present study was to use L-[2-^{15}N] glutamine to investigate the effects of standard TPN and TPN supplemented with glutamine (TPNGLN) on whole body glutamine metabolism in critically ill patients.

Subjects

Twelve severely ill patients (age range 32-76 years) in the ICU of St Thomas' Hospital were studied. Further details of their clinical and metabolic characteristics are summarised in Table 6.1. The majority of the patients had undergone emergency abdominal surgery within 24 hours of the start of the study. The study protocol is described in Chapter 2. At the end of the baseline study (Study 1) the patients were

Table 6.1. Characteristics of critically ill patients receiving TPN or TPNGLN

Subject No.	*Gender*	*Age (yrs)*	*Weight (kg)*	*Height (cm)*	*BMI ($kg\ m^{-2}$)*	*APACHE II*	*TISS*	*Plasma Albumin ($g\ l^{-1}$)*	*Plasma CRP ($mg\ l^{-1}$)*	*Main Diagnosis*	*28 Day Mortality*
TPN											
1	F	68	64.0	171	22.2	15	34	34	82	Bowel Resection	-ve
2	F	55	42.0	158	16.8	20	47	28	191	Bowel Resection	-ve
3	M	76	73.6	182	22.2	23	49	27	68	GI Bleed/laparotomy	-ve
4	M	74	66.0	175	21.6	20	48	18	217	AAA	-ve
5	F	69	60.5	162	23.1	18	42	25	185	AAA	-ve
6	M	73	63.0	170	21.8	19	49	22	212	AAA	-ve
mean ± SE		**69 ± 3**	**61.5 ± 4.3**	**170 ± 4**	**21.2 ± 0.9**	**19 ± 1**	**45 ± 2**				
TPNGLN											
1	M	72	82.0	187	23.4	15	37	23	161	GI Obstruction	-ve
2	F	65	75.0	170	26.0	9	50	19	557	Bowel Resection	-ve
3	M	75	103.7	183	31.0	24	47	27	203	AAA	-ve
4	F	75	85.0	168	30.1	19	38	23	329	Bowel Obstruction	-ve
5	M	59	98.0	181	29.9	14	47	20	247	Bowel Resection	-ve
6	F	32	58.5	170	20.2	18	62	17	145	Bowel Resection	-ve
mean ± SE		**63 ± 7**	**83.7 ± 6.6**	**177 ± 3**	**23.8 ± 4.1**	**17 ± 2**	**46 ± 3**				

BMI, body mass index; APACHE II, acute physiology and chronic health evaluation; TISS, therapeutic intervention scoring system; CRP, C-reactive protein; GI, gastrointestinal; AAA, aortic abdominal aneurysm; -ve, negative.

randomised to receive either standard TPN, or TPN with additional intravenous glutamine (TPNGLN: 0.4 g kg^{-1} day^{-1}, Oxford Nutrition, Oxford, UK). The standard TPN consisted of 50 % dextrose, Intralipid 20 % (Kabivitrum Inc., Stockholm, Sweden) and a mixed amino acid solution (Vamin 14; Pharmacia & Upjohn, Milton Keynes, UK). The composition of Intralipid 20 % and Vamin 14 are shown in Tables 6.2 and 6.3 respectively. This TPN combination provided 30 kCal kg^{-1} day^{-1} and 0.2 gN kg^{-1} day^{-1}. The patients randomised to TPNGLN received additional nitrogen in the form of the glutamine infusion (~ 0.06 gN kg^{-1} day^{-1}). The nutritional support was not made isonitrogenous as this could have limited the availability of other amino acids. A continuous insulin infusion (Actrapid; Novo Nordisk, Copenhagen, Denmark) was provided if necessary, as part of the clinical care, to maintain plasma glucose concentration at or below 7 mmol l^{-1}. Following 72 hours of nutritional support a second turnover study (Study 2) was performed, the TPN was maintained throughout this second study.

Table 6.2. Composition of Intralipid 20 %.

Ingredients	***Concentration per 500 ml***
Purified soybean oil	100 g
Fractionated egg phospholipids	6 g
Glycerol	11 g

Table 6.3. Composition of Vamin 14.

Ingredients	***Concentration per 1,000 ml***
L-Alanine	12.0 g
L-Arginine	8.4 g
L-Aspartic acid	2.5 g
L-Cysteine/cystine	420 mg
L-Glutamic acid	4.2 g
Glycine	5.9 g
L-Histidine	5.1 g
L-Isoleucine	4.2 g
L-Leucine	5.9 g
L-Lysine	6.8 g
L-Methionine	4.2 g
L-Phenylalanine	5.9 g
L-Proline	5.1 g
L-Serine	3.4 mg
L-Threonine	4.2 g
L-Tryptophan	1.4 g
L-Tyrosine	170 mg
L-Valine	5.5 g
Sodium	100 mmol
Potassium	50 mmol
Calcium	5 mmol
Magnesium	8 mmol
Chloride	100 mmol
Sulphate	8 mmol
Acetate	135 mmol

Results

Table 6.1 shows the details of the twelve critically ill patients studied. There were no significant differences in age, weight, height or BMI between the two treatment groups. The severity of illness is indicated by the APACHE II and TISS scores which identify the patients as being severely ill and dependent on cardiorespiratory and nutritional support (Cullen et al 1974, Knaus et al 1985). There were no significant differences in the APACHE II and TISS scores between the two groups of patients at the start of the study. None of the patients were acidaemic, there were no significant differences in the arterial pH values between the treatment groups in Study 1 (TPN 7.43 ± 0.03; TPNGLN 7.44 ± 0.02). In addition bicarbonate concentrations indicated no depletion of alkaline reserves (TPN 26 ± 2 mmol l^{-1}; TPNGLN 26 ± 1.5 mmol l^{-1}). There were no significant changes in blood gas analysis results between Study 1 and Study 2 for both patient groups. Plasma urea concentrations were elevated in both groups indicating high rates of protein catabolism (TPN 13 ± 2 mmol l^{-1}; TPNGLN 13 ± 2.5 mmol l^{-1}; normal reference range 4-7 mmol l^{-1}). The exogenous insulin infusion rates are shown in Table 6.4

The hormone and metabolite data for the patients receiving TPN and TPNGLN is summarised in Table 6.5. Plasma glucose levels increased significantly with treatment in the TPN group ($p<0.05$), but not in the TPNGLN group. Insulin levels increased significantly in Study 2 for both treatment groups (TPN, $p<0.01$; TPNLGLN, $p<0.05$). C-peptide levels were significantly increased in the second study in the TPNGLN group ($p<0.05$), but not in the TPN group. There were no significant changes in GH levels between Study 1 and 2 for both treatment groups. Total IGF-I was unchanged following both TPN and TPNGLN, however IGFBP-1 was significantly decreased in Study 2 in the TPN group ($p<0.05$). There were no significant changes in cortisol, glucagon, free thyroxine or free tri-iodothyroxine levels between Study 1 and 2 for both treatment groups.

Plasma amino acid profiles are shown in Table 6.6. Total amino acids were significantly increased in the second study in the patients receiving TPNGLN ($p<0.01$), but not in the patients receiving standard TPN. BCAA levels were unchanged by both treatments. In the group receiving TPN there were significant increases in plasma serine ($p<0.05$), glycine ($p<0.05$), alanine ($p<0.05$), methionine ($p<0.01$) and ornithine

($p<0.05$) levels in Study 2. Plasma serine ($p<0.05$), glutamate ($p<0.05$), glycine ($p<0.05$), alanine ($p<0.001$), methionine ($p<0.05$), and histidine ($p<0.01$) were all significantly increased in the second study in the patients receiving TPNGLN. Plasma glutamine concentrations were significantly increased by treatment with TPNGLN ($p<0.001$), but not by treatment with TPN (Figure 6.3).

Table 6.4. Exogenous insulin infusion rates for patients receiving TPN or TPNGLN.

Subject No.	***Insulin Infusion rate ($U\ hr^{-1}$)***	
	Study 1	***Study 2***
TPN		
1	2	2
2	0.5	2
3	0	0
4	0	1
5	1	1
6	0	0
TPNGLN		
1	0	1
2	0	3
3	1	3
4	0	0
5	2	2
6	0	0

Table 6.5. Hormone and metabolite concentrations of critically ill patients receiving TPN or TPNGLN. Values are means ± SE, *n=6*.

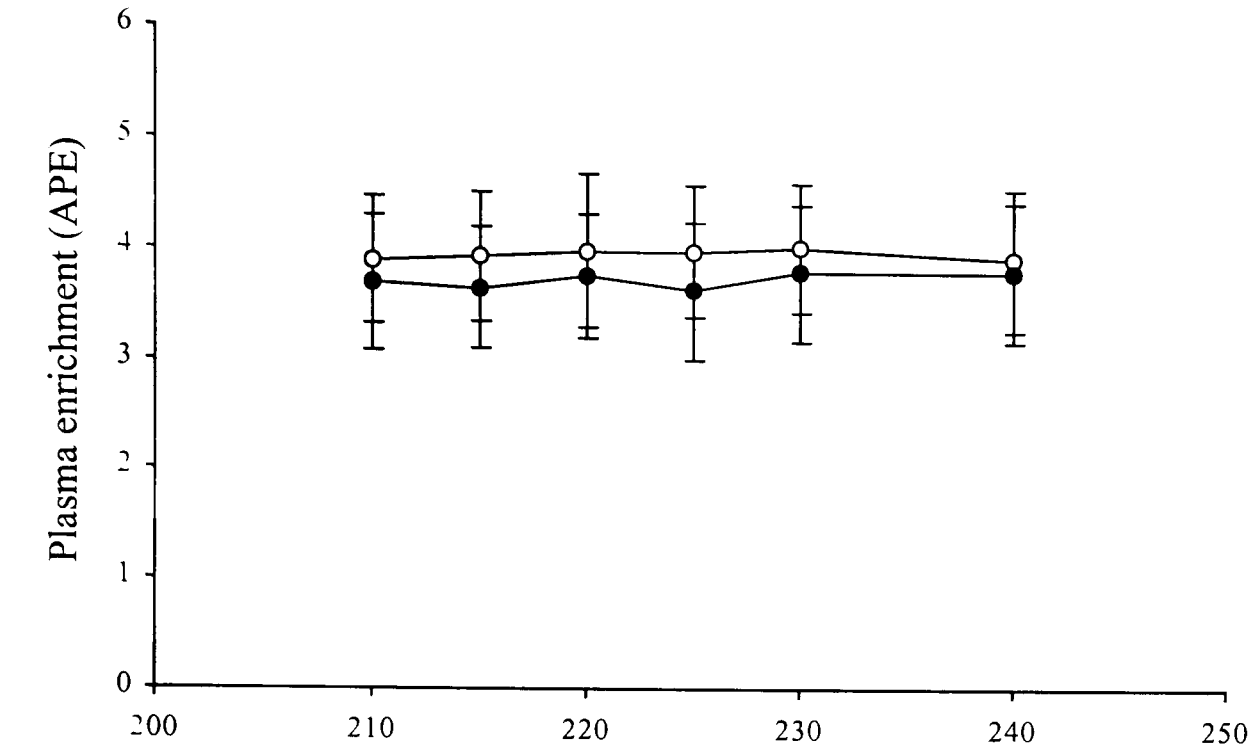
	Units	*TPN*			*TPNGLN*		
		Study 1	***Study 2***	***p***	***Study 1***	***Study 2***	***p***
Glucose	mmol l^{-1}	6.6 ± 0.5	7.8 ± 0.3	<0.05	6.3 ± 0.5	7.7 ± 0.5	NS
Insulin	mU l^{-1}	17.0 ± 2.9	53.8 ± 10.4	<0.01	18.5 ± 3.0	88.7 ± 36.0	<0.05
C-peptide	nmol l^{-1}	1.83 ± 0.49	2.11 ± 0.61	NS	1.45 ± 0.32	2.09 ± 0.41	<0.05
GH	mU l^{-1}	5.28 ± 1.81	6.98 ± 2.57	NS	2.81 ± 0.66	2.78 ± 1.13	NS
IGF-I	nmol l^{-1}	13.6 ± 1.8	11.0 ± 2.4	NS	12.1 ± 0.9	10.8 ± 1.0	NS
IGFBP-1	ng ml^{-1}	231 ± 79	39 ± 12	<0.05	87 ± 35	132 ± 86	NS
IGFBP-3	ng ml^{-1}	1116 ± 334	1250 ± 412	NS	1256 ± 153	1270 ± 108	NS
Cortisol	nmol l^{-1}	469 ± 53	486 ± 69	NS	872 ± 325	1231 ± 765	NS
Glucagon	pg ml^{-1}	383 ± 67	360 ± 145	NS	256 ± 47	304 ± 44	NS
Free Thyroxine	pmol l^{-1}	14.0 ± 1.9	13.6 ± 1.8	NS	10.8 ± 1.3	9.7 ± 1.6	NS
Free Tri-iodothyronine	pmol l^{-1}	3.9 ± 0.6	3.9 ± 0.6	NS	2.9 ± 0.5	3.1 ± 0.3	NS

Table 6.6. Plasma amino acid profiles of critically ill patients receiving TPN or TPNGLN. Values are means ± SE, *n=6*.

Amino acid	*TPN*			*TPNGLN*		
(μmol l^{-1})	*Study 1*	*Study 2*	*p*	*Study 1*	*Study 2*	*p*
Aspartate	34 ± 10	38 ± 9	NS	14 ± 6	20 ± 6	NS
Threonine	49 ± 8	91 ± 15	NS	57 ± 6	73 ± 8	NS
Serine	46 ± 4	105 ± 16	<0.05	54 ± 6	84 ± 13	<0.05
Glutamate	30 ± 8	94 ± 29	NS	36 ± 7	61 ± 14	<0.05
Glutamine	376 ± 34	426 ± 58	NS	338 ± 22	461 ± 24	<0.001
Glycine	153 ± 29	318 ± 68	<0.05	145 ± 13	220 ± 23	<0.05
Alanine	200 ± 35	414 ± 72	<0.05	170 ± 20	278 ± 26	<0.001
Valine	191 ± 29	222 ± 28	NS	158 ± 13	174 ± 17	NS
Cystine	50 ± 9	70 ± 17	NS	55 ± 14	50 ± 4	NS
Methionine	11 ± 2	27 ± 2	<0.01	17 ± 2	31 ± 6	<0.05
Isoleucine	36 ± 8	32 ± 4	NS	41 ± 6	42 ± 8	NS
Leucine	116 ± 19	112 ± 18	NS	98 ± 8	86 ± 9	NS
Tyrosine	41 ± 9	38 ± 7	NS	49 ± 7	36 ± 12	NS
Phenylalanine	80 ± 8	105 ± 9	NS	82 ± 10	105 ± 20	NS
Ornithine	32 ± 3	95 ± 22	<0.05	40 ± 5	72 ± 16	NS
Lysine	99 ± 12	144 ± 18	NS	105 ± 12	125 ± 11	NS
Histidine	41 ± 4	52 ± 4	NS	38 ± 5	61 ± 8	<0.01
BCAA	337 ± 54	365 ± 49	NS	290 ± 29	302 ± 31	NS
Total	1546 ± 149	2324 ± 309	NS	1463 ± 94	1967 ± 108	<0.01

The plasma glutamine enrichments and concentrations from both studies for the TPN patient group are shown in Figure 6.1 and for the TPNGLN in Figure 6.2. These figures show that a "steady state" was achieved during the final 30 minutes of tracer infusion. The glutamine turnover data for both groups of patients is summarised in Figure 6.3. The glutamine production rate was not affected by treatment with TPN (5.77 ± 0.49 vs. 5.50 ± 0.56 μmol min^{-1} kg^{-1}) or TPNGLN (5.06 ± 0.50 vs. 5.42 ± 0.67 μmol min^{-1} kg^{-1}). Glutamine uptake was unchanged in the TPN group (6.01 ± 0.51 vs. 5.70 ± 0.57 μmol min^{-1} kg^{-1}) but was significantly increased in the second study in the TPNGLN group (5.23 ± 0.50 vs. 7.41 ± 0.69 μmol min^{-1} kg^{-1}; $p<0.05$). MCR_{gln} was not affected by treatment with TPN (15.65 ± 1.02 vs. 14.23 ± 2.22 ml min^{-1} kg^{-1}) or TPNGLN (14.97 ± 1.06 vs. 15.55 ± 0.84 ml min^{-1} kg^{-1}).

B_{gln} was significantly decreased in Study 2 in the TPN group (2.48 ± 0.15 vs. 2.15 ± 0.19 μmol min^{-1} kg^{-1}; $p<0.01$) and although a similar trend was observed in the TPNGLN group this failed to reach significance (1.96 ± 0.11 vs. 1.66 ± 0.18 μmol min^{-1} kg^{-1}; $p=0.08$). When expressed as a percentage of the endogenous Ra_{gln}, protein derived glutamine release was decreased following treatment with both TPN (44 ± 4 vs. 40 ± 3 %; $p<0.05$) and TPNGLN (40 ± 3 vs. 32 ± 3 %; $p<0.05$) (Figure 6. 4). D_{Gln} was not significantly altered by treatment with TPN (3.29 ± 0.45 vs. 3.34 ± 0.46 μmol min^{-1} kg^{-1}) or TPNGLN (3.10 ± 0.45 vs. 3.76 ± 0.54 μmol min^{-1} kg^{-1}). However, the percentage of Ra_{gln} arising from de novo synthesis was significantly increased following treatment with TPN (56 ± 4 vs. 60 ± 3 %; $p<0.05$) and TPNGLN (60 ± 3 vs. 68 ± 3 %; $p<0.05$) (Figure 6.4).



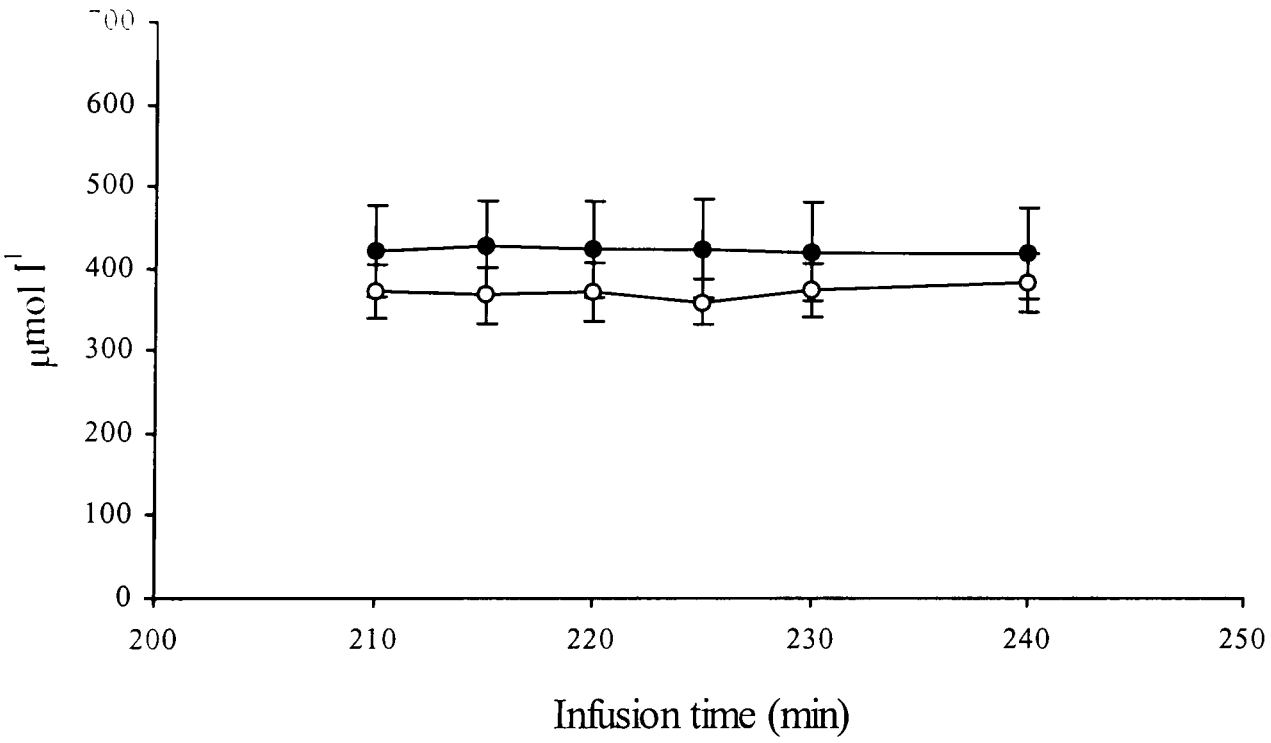
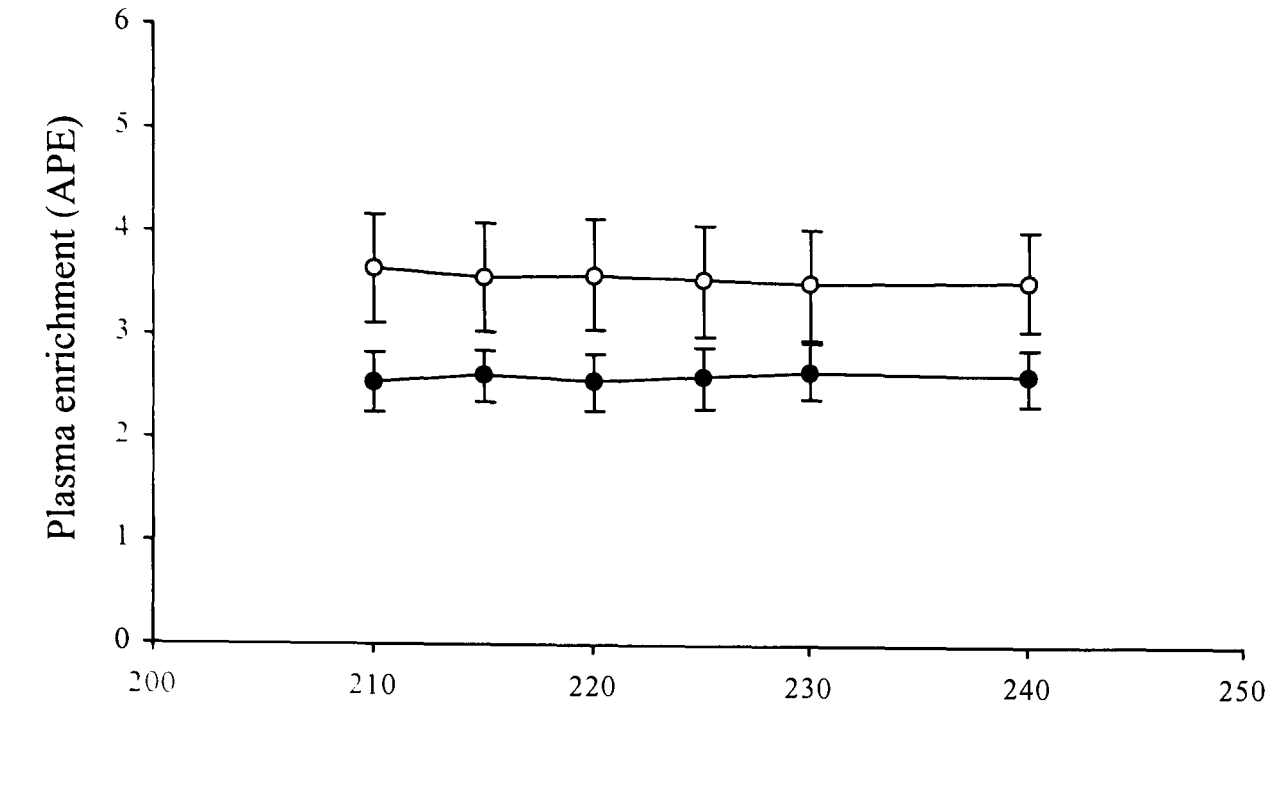


Figure 6.1. Plasma glutamine enrichment (APE) and concentration (μmol l^{-1}) for critically ill patients receiving standard TPN, Study 1 (○) and Study 2 (●). Values are means ± SE; *n*=6.



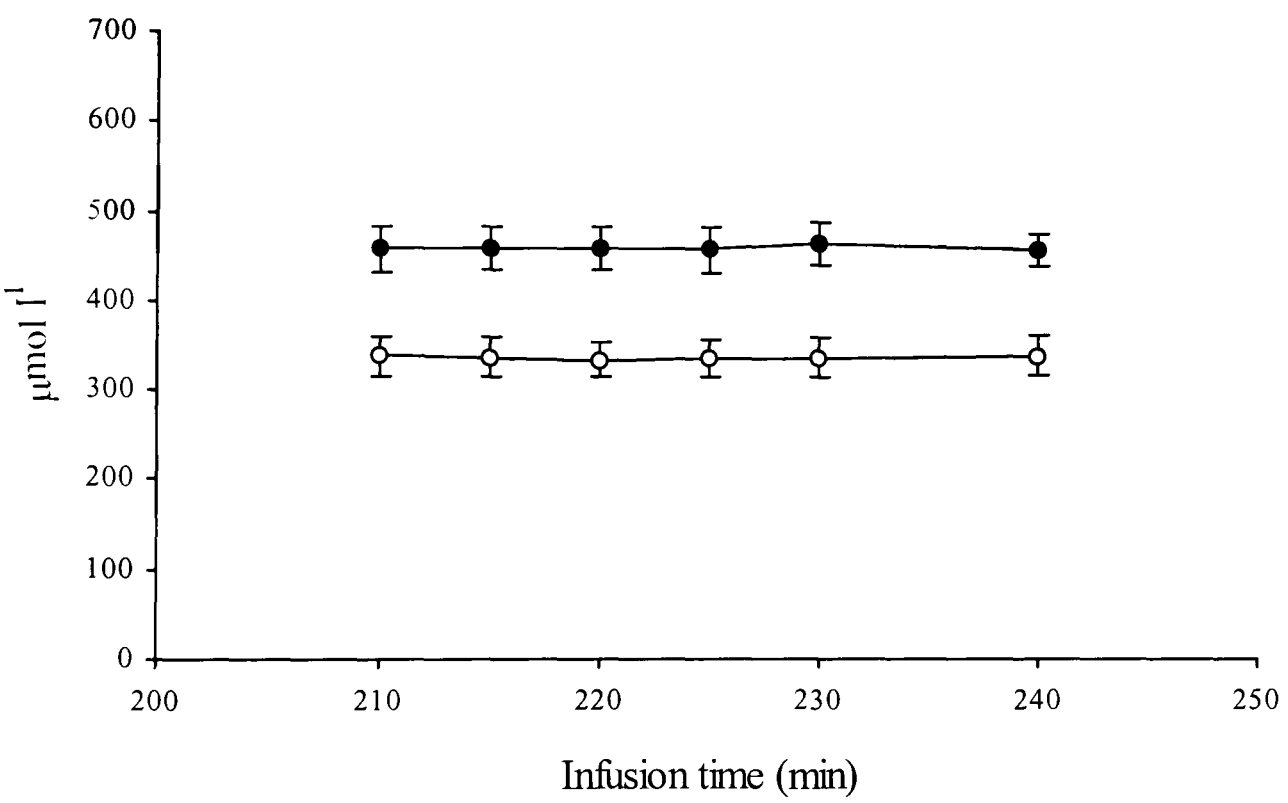
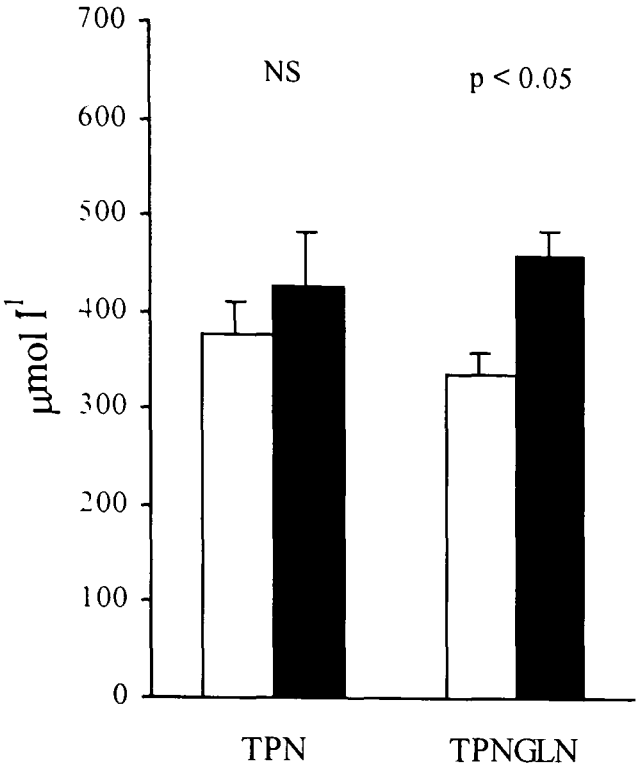
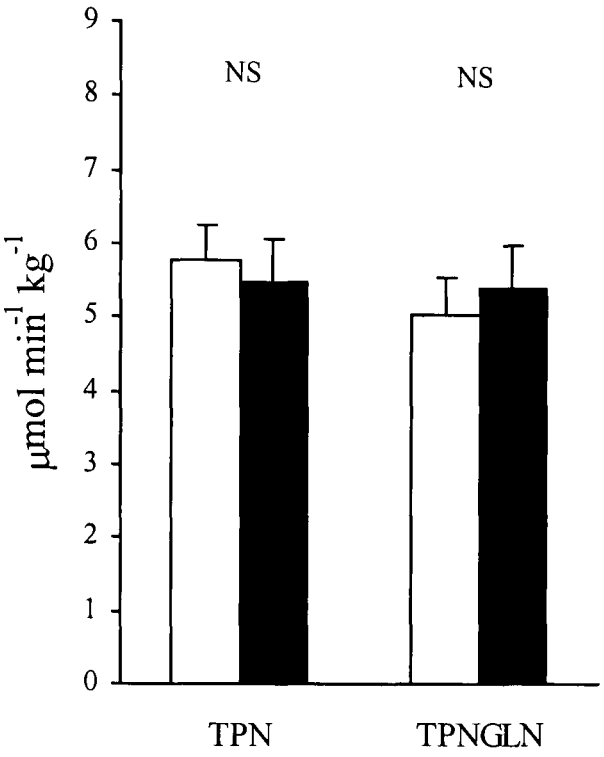


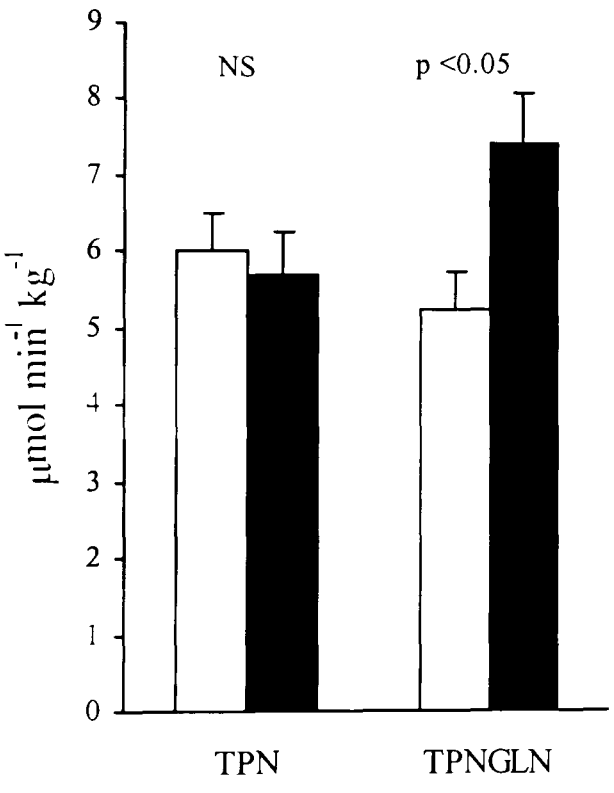
Figure 6.2. Plasma glutamine enrichment (APE) and concentration (μmol l^{-1}) for critically ill patients receiving TPNGLN, Study 1 (○) and Study 2 (●). Values are means ± SE; *n*=6.



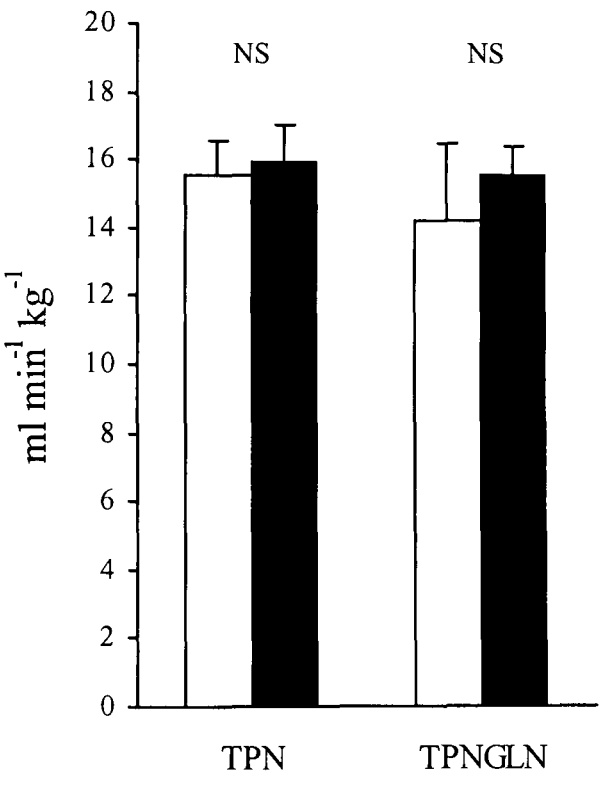
Plasma glutamine concentration



Endogenous glutamine Ra



Glutamine uptake



Glutamine MCR

Figure 6.3. Plasma glutamine concentration, glutamine appearance rate, uptake and metabolic clearance rate in critically ill patients receiving TPN or TPNGLN, Study 1 (□) and Study 2 (■). Values are means ± SE; *n*=6 in each group.

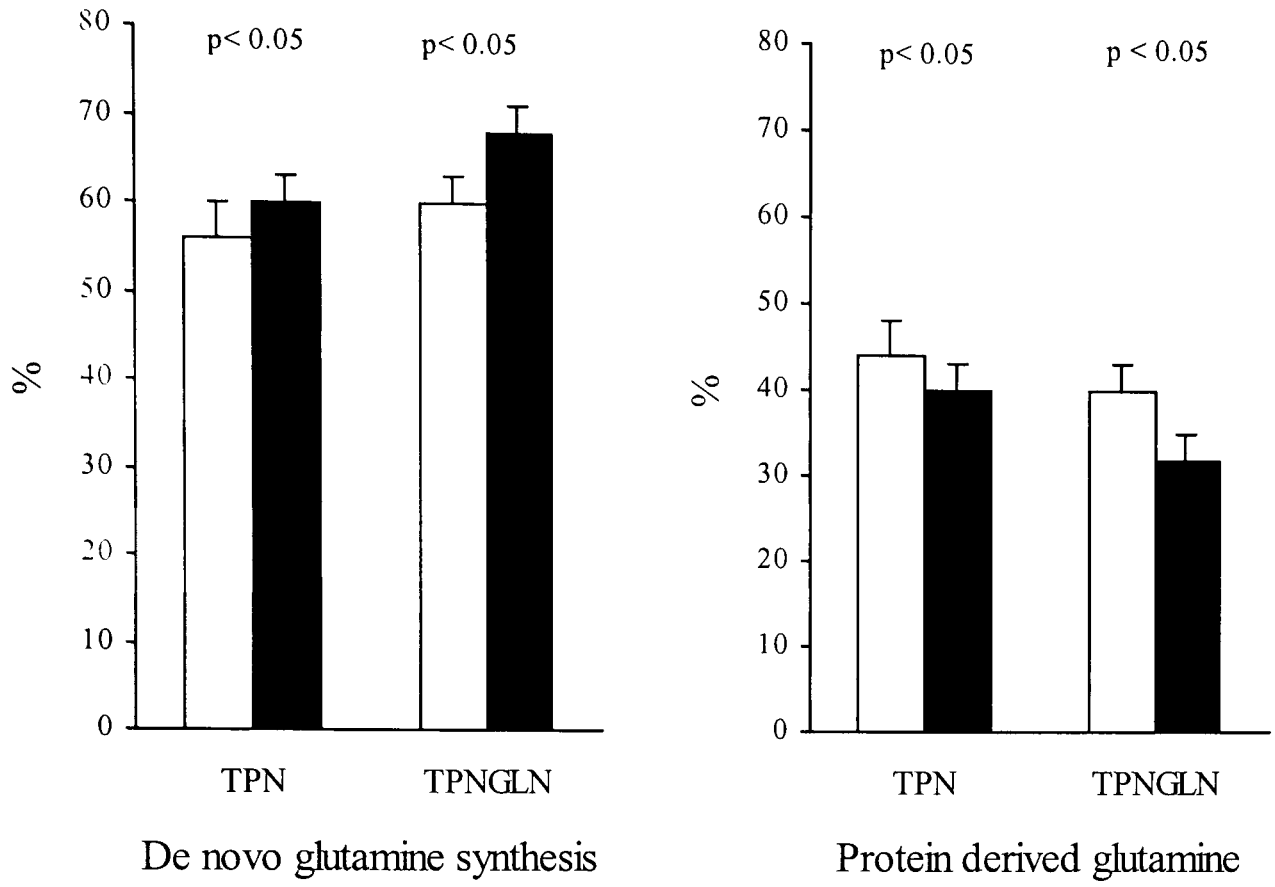


Figure 6.4. Percentage of endogenous glutamine appearance rate arising from de novo synthesis and proteolysis in critically ill patients receiving TPN or TPNGLN, Study 1 (□) and Study 2 (■). Values are means ± SE; *n*=6 in each group.

Discussion

Glutamine is considered to be a "conditionally" essential amino acid as during situations of severe stress both the circulating and intracellular free glutamine concentrations fall. Several studies have shown that the addition of glutamine or its analogues to TPN improves nitrogen balance, and reduces the fall in muscle glutamine concentration and protein synthesis in post-operative patients (Stehle et al 1989, Hammarqvist et al 1989, Wernerman et al 1990, Petersson et al 1994, Blomqvist et al 1995). In the present study we found glutamine supplementation to TPN increased plasma glutamine concentration. By using an isotopic tracer of glutamine to measure glutamine metabolism we were also able to demonstrate that whole body glutamine uptake was increased. This increased uptake may be for use by tissues with high metabolic demands for glutamine or to replenish the muscle free pools. TPN without glutamine failed to normalise glutamine concentration and had no effect on glutamine uptake suggesting that glutamine supplementation may be important to meet the increased metabolic demands of glutamine requiring tissues in the catabolic state.

In Chapter 5 it was shown that despite a marked decrease in plasma glutamine concentration, Ra_{gln} was unchanged in critically ill patients compared with matched healthy controls. It was also found that MCR_{gln} was increased in these patients, suggesting the primary mechanism for the reduced concentration may be an increased efficiency of glutamine transport. In the present study when nutritional support was provided, with or without glutamine supplementation, the glutamine production rate was unchanged and MCR_{gln} remained elevated. The increased glutamine uptake would thus have been facilitated by the increased efficiency of glutamine transport.

As glutamine is a non-essential amino acid the measured Ra_{gln} is derived from de novo glutamine synthesis and glutamine release from protein breakdown. In the present study there was a decrease in the proportion of endogenous glutamine production derived from protein breakdown (and an increase in de novo synthesis) when the patients were receiving nutritional support. Since glutamine production rate was unchanged in the present study this suggests that there was a shift to de novo synthesis of glutamine from the exogenous supply of amino acids. This may be related to the provision of substrates and energy for de novo synthesis or to the changes in the

patients' insulin levels. Exogenous insulin was given when necessary to maintain the patients' blood glucose at or below 7 mmol l^{-1}, a standard procedure in the ICU at St Thomas' Hospital. Plasma glucose levels increased to a similar extent in both patient groups when nutritional support was provided, however this increase only reached significance in the TPN group. C-peptide levels, a measure of endogenous insulin secretion, were higher in both groups of patients in Study 1 compared to the elderly normals described in Chapter 4 (0.48 ± 0.13 nmol l^{-1}). C-peptide levels increased in Study 2 in both groups indicating increased insulin secretion in response to feeding, but this increase was only significant in the TPNGLN treated patients. Insulin levels also tended to be higher in the critically ill patients compared to the elderly normals and were increased in Study 2 in both groups of patients. As can be seen from Table 6.2 the patients were receiving a wide range of exogenous insulin infusion rates (0-3 U hr^{-1}). This treatment contributed to the observed increase in plasma insulin levels in both treatment groups. Since insulin plays a central role in the control of protein metabolism by reducing protein breakdown (Gelfand and Barrett 1987) this may account for some of the decrease in glutamine production derived from protein breakdown. Similarly the insulin deficiency of type 1 diabetes has been shown to increase the proportion of the glutamine appearance from protein breakdown (Darmaun et al 1991b).

Marked decreases in free glutamine concentrations have been reported in catabolic states associated with protein wasting. In addition experiments using animal models had indicated a positive relationship between protein synthesis and glutamine concentration (MacLennan et al 1987, Jepson et al 1988). This led to interest in the use of glutamine supplementation in critically ill patients with the original hypothesis that glutamine supplementation would help counteract the muscle protein catabolism seen in these patients. Several groups have investigated the effects of supplementing TPN with glutamine, or its analogues on protein metabolism in post-operative patients.

Glutamine solutions with long term stability are now available and in the present study we used a glutamine solution (20 g in 800 mls) prepared by aseptic filtration from Oxford Nutrition which had a shelf life of 1 year when stored at –20 °C. However, two different approaches were previously taken to overcome the pharmaceutical difficulties of including glutamine in TPN. Firstly the glutamine solution was prepared, sterilised

by filtration and added to the TPN immediately before administration to the patients. Using this approach, Hammarqvist and co-workers evaluated the impact of 3 days of standard TPN and glutamine enriched TPN (0.285 g kg^{-1} day^{-1}) on protein metabolism in patients undergoing elective abdominal surgery (cholecystectomy) (Hammarqvist et al 1989). The cumulative nitrogen balance was less negative in the patients receiving glutamine supplementation. In the same study muscle biopsy samples were taken before and three days after the operation to measure the intracellular amino acids and also the concentration and size distribution of ribosomes (as a measure of muscle protein synthesis). In the patients receiving glutamine the decrease in intracellular free glutamine concentration was less severe, and the ribosomal analysis indicated that protein synthesis was maintained at pre-operative levels. While suitable for research purposes this method of glutamine administration is not practical for routine clinical use.

The second approach to include glutamine in TPN was to utilise more stable glutamine analogues such as the glutamine containing dipeptides (Stehle et al 1989, Hammarqvist et al 1990). Stehle et al 1989 reported an improved nitrogen balance, which was associated with maintenance of the intracellular glutamine pools, in post-operative patients receiving TPN supplemented with the dipeptide L-alanyl-L-glutamine. Hammarqvists group reported similar results to their earlier study (preservation of muscle free glutamine and muscle protein synthesis and improved nitrogen balance) when the TPN was supplemented with L-alanyl-L-glutamine instead of glutamine (Hammarqvist et al 1990). A reduction in the loss of muscle free glutamine has also been reported in studies using ornithine-α-ketoglutarate, or α-ketoglutarate as precursors of glutamine to supplement TPN (Wernerman et al 1989, 1990). Petersson and co-workers looked at the longer-term effects of glutamine supplementation in patients undergoing elective abdominal surgery (Petersson et al 1994). Patients were randomised to receive either standard TPN or TPN supplemented with the dipeptide glycyl-glutamine for the first three days following surgery. Oral food was started on the third day post operatively. In the patients receiving TPN the muscle free glutamine levels were decreased by day 3, this decrease was delayed until day 10 in the patients receiving glutamine supplementation. These results suggest that glutamine supplementation maintains intracellular glutamine concentrations but if the glutamine supplementation is discontinued the muscle free glutamine content falls.

These early studies on the possible benefits of glutamine supplementation measured nitrogen balance and protein synthesis using ribosomal analysis, for example, a co-variation in the change in muscle glutamine concentration and polyribosome content was reported in post-operative patients (Wernerman et al 1990). The use of ribosomal analysis as a measure of protein synthesis has been questioned and this technique is now considered a less robust index of protein synthesis than a change in protein synthesis measured directly (Smith and Rennie 1996). The improvement in nitrogen balance with glutamine supplementation led to the assumption that some of the benefits associated with glutamine supplementation were mediated through alterations in protein metabolism and the preservation of muscle mass. However, it has been suggested that the interpretation of the improvement in nitrogen balance needs caution as a significant proportion of this balance may be due to replenishment of the muscle glutamine pool rather than increased protein synthesis (Walser 1991). In the present study (results not reported) glutamine supplementation had no additional effects on whole body protein breakdown, synthesis or balance (Carroll et al 1999).

Using [1-^{14}C] leucine to measure in vivo protein metabolism Hankard et al (1996) investigated the acute effects of a 5 hour enteral infusion of glutamine (800 μmol kg^{-1} h^{-1}) on whole body protein metabolism in healthy adults. Doubling the plasma glutamine concentration (582 ± 13 vs. 1,184 ± 37 μmol l^{-1}) decreased leucine oxidation (~42 %) and increased non-oxidative leucine disposal, a measure of whole body protein synthesis (~7 %). In contrast phenylbutyrate induced short-term (24 hour) plasma glutamine depletion in normal subjects (380 ± 15 vs. 514 ± 24 μmol l^{-1}) increased leucine oxidation (~22 %) and decreased protein synthesis (~11 %) (Darmaun et al 1998). These studies suggest glutamine may play a role in the regulation of whole body protein synthesis in healthy adults (Darmaun et al 1998). However, there is no direct evidence from studies in humans that increased muscle glutamine concentration enhances protein synthesis in catabolic states. In a study using [1-^{13}C] leucine to measure protein metabolism in children with Duchenne Muscular Dystrophy, an acute (5 hour) oral load of glutamine (800 μmol kg^{-1} h^{-1}) which doubled the plasma glutamine concentration (481 ± 16 vs. 804 ± 85 μmol l^{-1}) decreased protein

breakdown (~8 %), but did not alter protein synthesis (Hankard et al 1998). Long and co-workers investigated the effects of 3 days of glutamine supplementation ($0.35\ g\ kg^{-1}\ day^{-1}$) of enteral nutrition on vivo protein metabolism in trauma patients and could not demonstrate any differences in protein synthesis, breakdown or nitrogen balance compared to patients receiving unsupplemented enteral nutrition. (Long et al 1995).

Therefore, it has not been firmly established whether the fall in free muscle glutamine concentration observed in catabolic states is a reflection of the protein catabolic state or a causative factor. The relationship between muscle glutamine concentration and protein synthesis could be co-incidental, with variables being linked by a common third factor such as insulin, or thyroid hormones (Jepson et al 1988).

The benefits of glutamine supplementation may be mediated through other tissues and pathways rather than a direct effect on muscle and protein. Glutamine is an important energy substrate for enterocytes (Powell-Tuck 1993). Supplementation of TPN with glutamine containing dipeptides has been shown to prevent the mucosal atrophy and increased intestinal permeability associated with long-term TPN (Van der Hulst et al 1993, Tremel et al 1994). The integrity of the gastrointestinal tract normally provides an important barrier to the entry of bacteria or toxins (Griffiths et al 1997). This effect of glutamine supplementation may partly explain the decrease in the number of infections, reduced hospital stay and costs which have been reported in bone marrow transplant patients receiving TPN supplemented with glutamine (Ziegler et al 1992, MacBurney et al 1994).

Glutamine is used at a high rate by immunocytes, and the low plasma glutamine levels seen in critically ill patients may contribute to immunosuppression (Parry-Billings et al 1990). In bone marrow transplant patients glutamine supplementation has been shown to increase the percentage of lymphocytes and improve the T lymphocyte population (Ziegler et al 1998). Glutamine is also an important precursor of the antioxidant glutathione. Intravenous glutamine has been shown to preserve plasma, liver and intestinal mucosal glutathione stores in animal models (Cao et al 1998, Hong et al 1992, Harward et al 1994).

Many of the early clinical studies of glutamine supplementation have studied patients undergoing elective surgery. These are homogenous groups of patients undergoing standard operative traumas, and are generally less severely ill than patients admitted to the ICU. In a more recent study in severely ill patients Palmer and colleagues found supplementing TPN with 25 g day^{-1} glutamine for 5 days did not produce a consistent pattern of change in either plasma or muscle free glutamine levels(Palmer et al 1996). Although we used a similar level of glutamine supplementation and the patients had a similar initial plasma glutamine concentration in the present study (~28 g day^{-1}) we were able to show a significant increase in plasma glutamine levels. In the study reported by Palmer and colleagues TPN was commenced ~3 days after the patients admission to the ICU, therefore these patients may have been more severely glutamine depleted than the patients in the present study where TPN was started within 1-2 days of admission

The nutritional support in the present study was not isonitrogenous since the patients receiving TPNGLN were given extra nitrogen in the glutamine supplementation (~0.06 gN kg^{-1} day^{-1}). We decided not to make the nutritional support isonitrogenous as we were concerned that to do so could have limited the availability of other amino acids. However, the changes in plasma amino acid profiles were similar in both groups and many of the amino acids were restored to near normal levels. The patients we have studied were recruited in the ICU from patients in whom the clinical decision had been made to use TPN. We chose to study these patients as they represent the group in whom there is considerable clinical interest in the potential benefits of glutamine supplementation, but they are a very heterogeneous group as can be seen from the patient details in Table 6.1. The changes in amino acid levels did not reach the same degree of statistical significance in the two treatment groups due to the wide variation in amino acid levels between the patients and the small patient numbers.

It was shown in the previous chapter that glutamine uptake is maintained in the critically ill, despite low glutamine concentrations, by an increase in the efficiency of glutamine transport as demonstrated by the increase in MCR_{gln} (Chapter 5). This is probably due to the increased metabolic demand for glutamine by tissues such as the immune system and the gastrointestinal tract in the catabolic state. In the present study we have shown that following glutamine supplementation glutamine concentration was

restored to normal but glutamine uptake was increased providing further evidence that glutamine may be a conditionally essential amino acid. Since MCR_{gln} remained elevated this suggests that an increased efficiency of glutamine transport facilitates the increased uptake of glutamine.

Chapter 7

EFFFECT OF GH/IGF-I THERAPY ON GLUTAMINE METABOLISM IN CRITICAL ILLNESS

Introduction

The availability of recombinant human GH and IGF-I has led to considerable interest in their use, either alone or in combination, to reduce protein catabolism, in a variety of catabolic states. However, as glutamine is mobilised from protein stores (skeletal muscle) during periods of illness, an increase in protein synthesis may lead to further glutamine depletion in critically ill patients (Hinds 1999).

Aim

The aim of the present study was to use L-[2-^{15}N] glutamine to investigate the effects of the addition of combined treatment with GH and IGF-I to TPN supplemented with glutamine (TPNGLN+GH/IGF-I) on whole body glutamine metabolism in critically ill patients.

Subjects

Five severely ill patients (age range 50-78 years) in the ICU of St Thomas' Hospital were studied, details of their clinical and metabolic characteristics are summarised in Table 7.1. Three of the patients had undergone emergency abdominal surgery within 24 hours of the start of the study, one patient had suffered a cardiac arrest and one had been admitted with a bowel obstruction. The study protocol is described in Chapter 2. At the end of the baseline study (Study 1) the patients received TPN with additional intravenous glutamine (0.4 g kg^{-1} day^{-1}) and combined rhGH (0.2 IU kg^{-1} day^{-1}) and rhIGF-I (160 μg kg^{-1} day^{-1}) therapy (TPNGLN+GH/IGF-I). The GH was administered as a single subcutaneous injection at 12:00 hours. The IGF-I dose was split into two equal twice daily subcutaneous injections at 12:00 and 22:00 hours. A continuous insulin infusion (Actrapid; Novo Nordisk, Copenhagen, Denmark) was provided if necessary, as part of the clinical care, to maintain plasma glucose concentration at or below 7 mmol l^{-1}. Following 72 hours of nutritional/hormonal support a second turnover study (Study 2) was performed, the TPN was maintained throughout this second study.

The control group for this study was a group of six critically ill patients who received TPNGLN (results presented in Chapter 6).

Results

Table 7.1 shows the details of the five critically ill patients receiving TPNGLN+GH/IGF-I. There were no significant differences in age, weight, height or BMI between the patients receiving TPNGLN+GH/IGF-I or the patients receiving TPNGLN described in Chapter 6. The severity of illness is indicated by the APACHE II and TISS scores which identify the patients as being severely ill and dependent on cardiorespiratory and nutritional support (Cullen et al 1974, Knaus et al 1985). There were no significant differences in the APACHE II and TISS scores between the two groups of critically ill patients at the start of the study. None of the patients in the TPNGLN+GH/IGF-I group were acidaemic (arterial pH values 7.39 ± 0.02) and bicarbonate concentrations indicated no depletion of alkaline reserves (25 ± 1.2 mmol l^{-1}). There were no significant changes in blood gas analysis results between Study 1 and Study 2. Plasma urea concentrations were elevated in Study 1 indicating high rates of protein catabolism (TPNGLN+GH/IGF-I 9.3 ± 1.4 mmol l^{-1}; normal reference range 4-7 mmol l^{-1}). None of the patients in the TPNGLN+GH/IGF-I group were given exogenous insulin during Study 1, and only 1 patient (patient 5) received 2 U hr^{-1} during Study 2.

The hormone and metabolite data for the patients receiving TPNGLN+GH/IGF-I is summarised in Table 7.2. Plasma glucose levels increased significantly in the second study ($p<0.05$). There were no significant changes in the plasma insulin or C-peptide levels between the two studies. Although there was a marked increase in GH levels in Study 2 this did not achieve statistical significance ($p=0.084$). As anticipated there was a significant increase in total IGF-I levels in Study 2 ($p<0.05$) to just above the normal range (41- >60 years: 3.7-43.9 nmol l^{-1}). IGFBP-3 levels were also significantly increased in Study 2 ($p<0.05$). There were no significant changes in IGFBP-1, cortisol, glucagon, or thyroid hormone levels between Study 1 and 2.

Plasma amino acid profiles are shown in Table 7.3. The plasma amino acid profiles were similar in Study 1 and 2, apart from significant increases in glutamine ($p<0.05$) and phenylalanine ($p<0.05$) concentrations.

Figure 7.1 shows the plasma glutamine enrichments and concentrations from Study 1 and 2 for the TPNGLN+GH/IGF-I patient group, during the final 30 minutes of tracer infusion indicating "steady state" was achieved. The glutamine turnover data is summarised in Figures 7.2 and 7.3. The glutamine turnover data from the TPNGLN group has been included in these figures for reference. Glutamine production rate and MCR_{gln} were not altered by treatment with TPNGLN+GH/IGF-I (5.08 ± 1.10 vs. 5.78 ± 0.82 μmol min^{-1} kg^{-1} and 16.92 ± 2.00 vs. 14.90 ± 1.58 ml min^{-1} kg^{-1}). Glutamine uptake was significantly increased in the second study in the TPNGLN+GH/IGF-I treated patients (5.2 ± 1.1 vs. 7.6 ± 0.8 μmol min^{-1} kg^{-1}; $p<0.05$).

B_{gln} and D_{gln} were not significantly altered by treatment with TPNGLN+GH/IGF-I (2.11 ± 0.25 vs. 1.91 ± 0.15 μmol min^{-1} kg^{-1} and 2.97 ± 1.00 vs. 3.86 ± 0.72 μmol min^{-1} kg^{-1}, respectively). When expressed as a percentage of the endogenous Ra_{gln}, the decrease in B_{gln} (46 ± 6 vs. 35 ± 4 %; p=0.08) and the increase in D_{gln} (54 ± 6 vs 65 ± 4 %; p=0.08) did not reach significance in this patient group (Figure 7.3).

Table 7.1. Characteristics of critically ill patients receiving TPNGLN+GH/IGF-I.

Subject No.	*Gender*	*Age (yrs)*	*Weight (kg)*	*Height (cm)*	*BMI ($kg\ m^{-2}$)*	*APACHE II*	*TISS*	*Plasma Albumin ($g\ l^{-1}$)*	*Plasma CRP ($mg\ l^{-1}$)*	*Main Diagnosis*	*28 Day Mortality*
1	M	65	65.0	182	19.7	19	50	15	378	Bowel Resection	-ve
2	F	54	61.0	171	20.9	20	52	11	105	Bowel Resection	+ve
3	M	50	60.0	179	18.7	9	38	26	38	GI Obstruction	-ve
4	M	62	115.0	179	35.9	16	42	20	249	Cardiac Arrest	-ve
5	M	78	76.0	175	24.8	14	47	24	361	AAA	-ve
mean ± SE		**62 ± 5**	**75.4 ± 10.3**	**177 ± 2**	**24.0 ± 3.1**	**16 ± 2**	**46 ± 3**				

BMI, body mass index; APACHE II, acute physiology and chronic health evaluation; TISS, therapeutic intervention scoring system; CRP, C-reactive protein; GI, gastrointestinal; AAA, aortic abdominal aneurysm; -ve, negative; +ve, positive.

Table 7.2. Hormone and metabolite concentrations of critically ill patients receiving TPNGLN+GH/IGF-I. Values are means ± SE, *n*=5.

	Units	*Study 1*	*Study 2*	*p*
Glucose	mmol l^{-1}	6.3 ± 1.0	8.3 ± 1.5	< 0.05
Insulin	mU l^{-1}	18.4 ± 5.0	58.5 ± 32.2	NS
C-peptide	nmol l^{-1}	1.33 ± 0.49	1.64 ± 0.52	NS
GH	mU l^{-1}	8.75 ± 4.34	29.58 ± 9.71	NS
IGF-I	nmol l^{-1}	10.3 ± 0.8	48.1 ± 9.1	< 0.05
IGFBP-1	ng ml^{-1}	134 ± 59	58 ± 15	NS
IGFBP-3	ng ml^{-1}	1466 ± 145	2749 ± 447	<0.05
Cortisol	nmol l^{-1}	411 ± 95	775 ± 341	NS
Glucagon	pg ml^{-1}	247 ± 82	316 ± 81	NS
Free Thyroxine	pmol l^{-1}	12.2 ± 2.8	15.3 ± 3.8	NS
Free Tri-iodothyronine	pmol l^{-1}	3.0 ± 0.3	4.2 ± 0.6	NS

Table 7.3. Plasma amino acid profiles of critically ill patients receiving TPNGLN+GH/IGF-I. Values are means ± SE, *n=5*.

Amino acid (mmol l^{-1})	*Study 1*	*Study 2*	*p*
Aspartate	18 ± 5	28 ± 12	NS
Threonine	57 ± 7	67 ± 10	NS
Serine	57 ± 9	73 ± 14	NS
Glutamate	43 ± 26	48 ± 10	NS
Glutamine	307 ± 65	524 ± 71	<0.05
Glycine	162 ± 29	265 ± 63	NS
Alanine	183 ± 42	286 ± 83	NS
Valine	148 ± 24	165 ± 23	NS
Cystine	37 ± 5	49 ± 8	NS
Methionine	14 ± 2	22 ± 6	NS
Isoleucine	27 ± 4	30 ± 3	NS
Leucine	84 ± 15	79 ± 12	NS
Tyrosine	62 ± 22	36 ± 4	NS
Phenylalanine	65 ± 7	82 ± 8	<0.05
Ornithine	35 ± 9	36 ± 7	NS
Lysine	115 ± 15	113 ± 16	NS
Histidine	46 ± 7	54 ± 8	NS
BCAA	260 ± 40	274 ± 33	NS
Total	1434 ± 161	1894 ± 270	NS

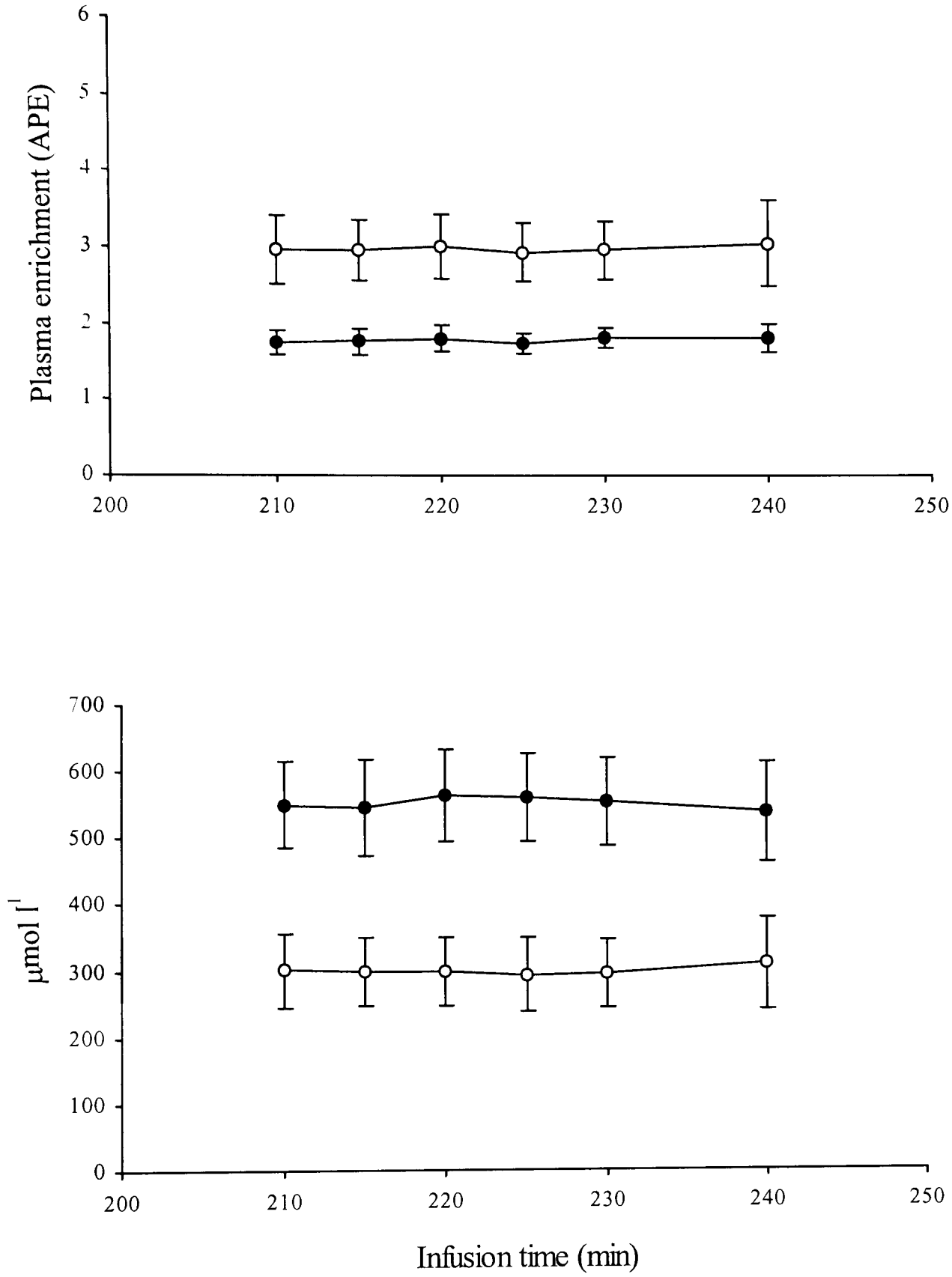


Figure 7.1. Plasma glutamine enrichment (APE) and concentration (µmol l^{-1}) for critically ill patients receiving standard TPNGLN+GH/IGF-I, Study 1 (○) and Study 2 (●). Values are means ± SE; $n = 5$.

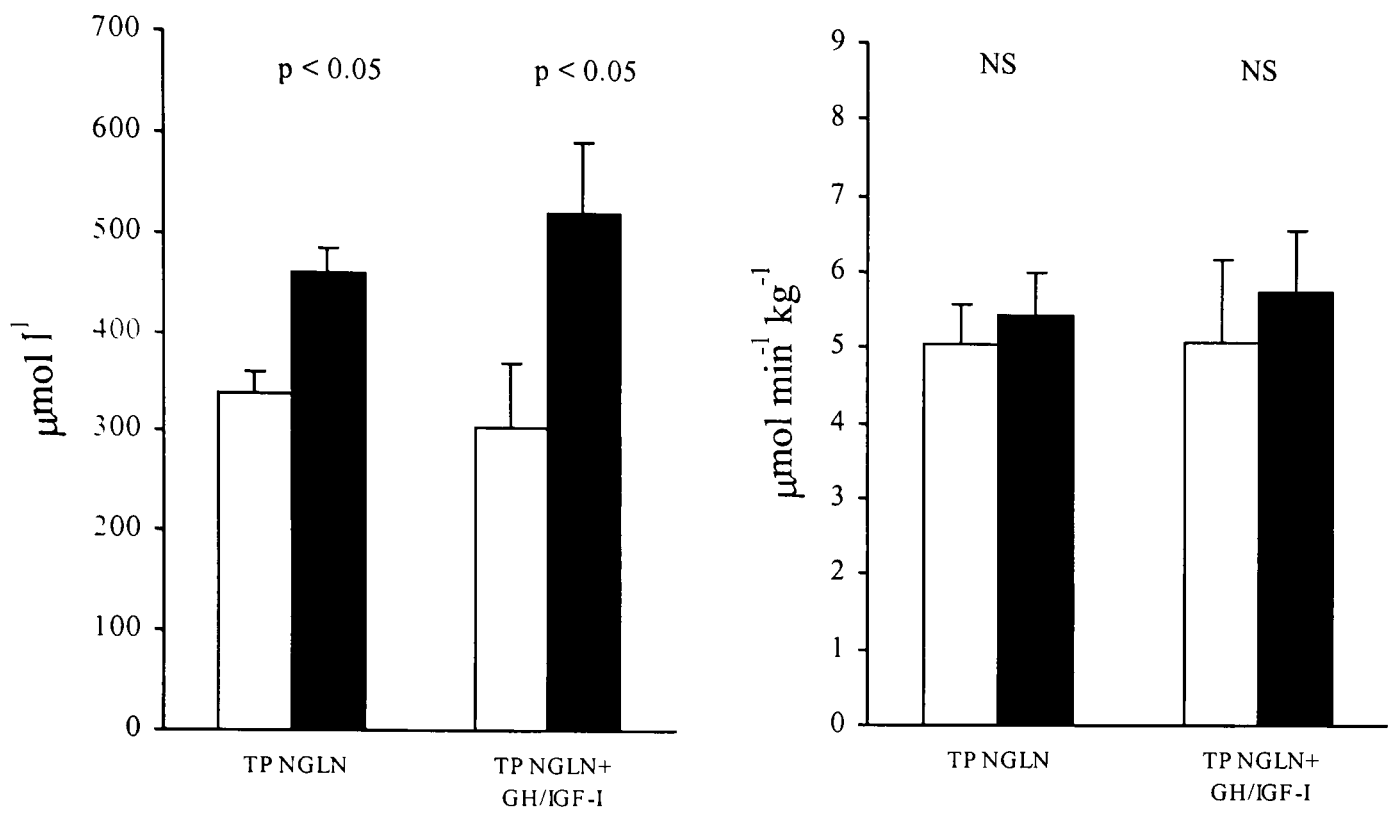


Figure 7.2. Plasma glutamine concentration, glutamine appearance rate, uptake and metabolic clearance rate in critically ill patients receiving TPNGLN (*n*=6) or TPNGLN+GH/IGF-I (*n*=5), Study 1 (□) and Study 2 (■). Values are means ± SE.

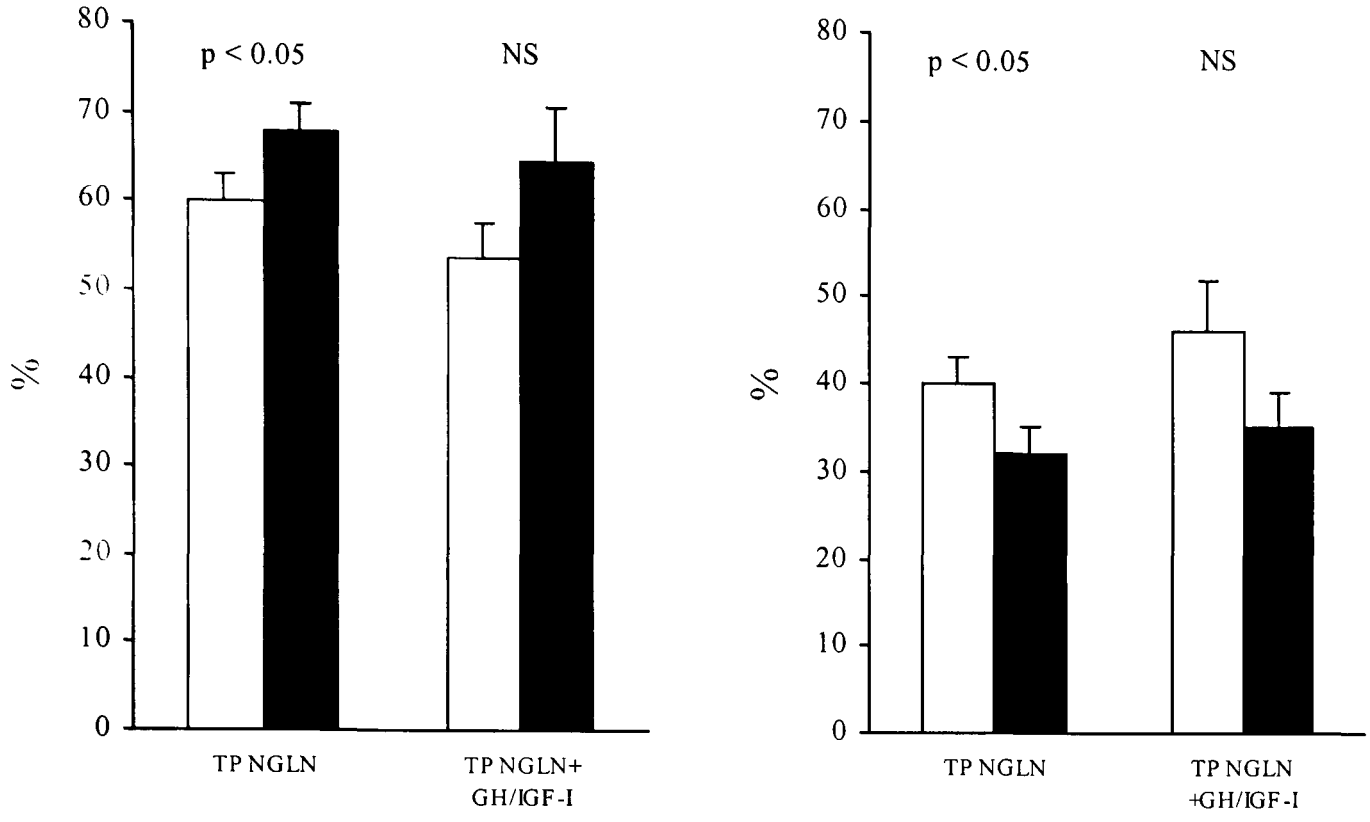


Figure 7.3. Percentage of endogenous glutamine appearance rate arising from de novo synthesis and proteolysis in critically ill patients receiving TPNGLN (*n*=6) or TPNGLN+GH/IGF-I (*n*=5), Study 1 (□) and Study 2 (■). Values are means ± SE.

Discussion

This study demonstrates that addition of combined GH/IGF-I therapy to nutritional support with glutamine supplementation in critically ill patients did not affect the increase in whole body glutamine uptake or the restoration of plasma glutamine concentration. GH treatment may reduce the availabity of glutamine for immunocytes, enterocytes and hepatic glutathione synthesis by preventing the moblization of glutamine from muscle (Takala et al 1999, Hinds 1999). The results of the present study suggest that combined GH/IGF-I therapy does not have adverse effects on glutamine metabolism these patients.

The continued loss of lean tissue in the critically ill despite enteral or parenteral nutritional support has led to interest in the use of anabolic hormones such as insulin, GH and IGF-I in these patients. The changes in the GH/IGF-I axis in critically ill patients are characterised as acquired GH resistance. Basal GH levels are increased, but pulsatile GH secretion is reduced, GH binding activity is reduced, IGF-I levels are low and IGFBP-1 levels are high (Ross et al 1991a, Ross et al 1991b). Both low and high affinity GH binding proteins (GHBP) have been identified, the high affinity GHBP represents the extracellular domain of the GH receptor and is thought to reflect GH receptor expression or abundance (Baumann 1999). The low levels of IGF-I reported in critically ill patients may be related to reduced GH receptor activity, reflected by the low GH binding activity (Ross et al 1991b). These changes may represent an adaptive change in critically ill patients reducing both any direct and the indirect effects of GH (anabolism and protein synthesis mediated by IGF-I) however this may be inappropriate in modern ICUs where nutritional support is available (Hinds 1999).

The effects of GH on protein metabolism have been demonstrated in GH deficient adults. GH replacement therapy increases lean body mass, and computerised topographic analysis of thigh cross sections suggest this increase is partly due to increased muscle content (Cuneo et al 1991). Whole body protein synthesis, measured by [1-^{13}C] leucine kinetics, was increased by ~ 24 % following two months of treatment (Russell-Jones et al 1993). However days or weeks of GH treatment increases the circulating IGF-I and insulin levels making it difficult to assess the direct action of GH on protein metabolism (Fryburg and Barrett 1995). In healthy adults a

6 hour infusion of GH (0.014 $\mu g\ kg^{-1}\ min^{-1}$) into the brachial artery stimulated forearm muscle protein synthesis by ~70 %, based upon the measurement of [^{3}H] phenylalanine extraction (Fryberg et al 1991). The systemic levels of GH, IGF-I or insulin were not affected by the local GH infusion indicating that GH itself was responsible for these changes. Copeland and Nair (1994) have also investigated the acute effects of GH on protein metabolism in healthy subjects in protocol designed to control for confounding perturbations in other hormones by using a simultaneous somatostatin infusion. Control subjects received replacement doses of insulin, glucagon, and GH. In the GH group the GH dose was increased to raise the GH levels to the high physiological range (12-20 $ng\ ml^{-1}$) for the final 3.5 hours of the protocol. GH treatment reduced leucine oxidation ($p=0.04$) and increased whole body protein synthesis ($p=0.07$), but there was no stimulation of muscle protein synthesis. This lack of stimulation of muscle protein synthesis by GH may have been due to the fact that the GH treatment period was too short to stimulate local production of IGF-I in muscle (Copeland and Nair 1994).

GH treatment has been shown to improve nitrogen balance in healthy volunteers receiving hypocaloric parenteral nutrition (Manson and Wilmore 1986), in patients (GI surgery, GI obstruction, fistulas or acute pancreatitis) established on parenteral nutrition (Ziegler et al 1988, Ponting et al 1990) and parenterally or enterally fed ICU patients (Ziegler et al 1990b). Improvement of nitrogen balance is assumed to result in a preservation of muscle mass, but these improvements can be brought about by an increase in whole body protein synthesis, a decrease in protein breakdown or a combination of the two. A number of studies have used isotope tracer studies to assess the effects of combining GH treatment with parenteral nutrition on protein metabolism in catabolic patients. Douglas et al (1990) suggested that the improved nitrogen balance following 3 days of GH treatment (20 $IU\ day^{-1}$, subcutaneous) in patients with sepsis and/or trauma resulted from increased protein synthesis rather than reduced breakdown. In severe trauma patients 7 days of treatment with GH (0.15 $mg\ kg^{-1}\ day^{-1}$, intramuscular) improved N balance and increased protein synthesis (Jeevandam et al 1996). In elderly patients (70 ± 4 years) 6 days of GH treatment (0.15 $IU\ kg^{-1}\ day^{-1}$, subcutaneous) following elective abdominal surgery, reduced leucine oxidation and increased whole body protein synthesis as assessed by an infusion of [1-^{13}C] leucine (Carli et al 1997). More recently Berman et al 1999 used [1-^{14}C] leucine and [2,6-^{3}H] phenylalanine to assess the effects of GH treatment (0.1 $mg\ kg^{-1}\ day^{-1}$) on whole body

and forearm skeletal muscle protein kinetics in patients who had undergone surgery for upper gastrointestinal malignancies. Combined treatment with TPN and GH significantly increased whole body net protein balance compared to TPN alone, but there was no significant effect on either whole body protein synthesis or breakdown. GH treatment tended to increase skeletal muscle protein synthesis and decrease protein breakdown resulting in a positive net protein balance, but these changes did not achieve significance.

The demonstration that GH had protein anabolic effects in catabolic patients led to two European multi-centre, trials being conducted in ICU patients to investigate the effects of GH treatment on the length of stay in the ICU and hospital, duration of mechanical ventilation, and outcome (Takala et al 1999). The patients studied had been in the ICU for 5-7 days and were expected to require intensive care for at least 10 more days. A total of 532 patients were given GH at a mean daily dose of 0.10 ± 0.02 mg kg^{-1} day^{-1} (0.3 ± 0.06 IU kg^{-1} day^{-1}) or placebo. However, rather than improving mortality as was expected, GH was found to significantly increase the in-hospital mortality rate in both studies ($p < 0.001$). In the patients who survived the duration of mechanical ventilation, intensive care and hospitalisation were prolonged by GH treatment. The precise mechanism(s) for the increased mortality and morbidity in these patients remain(s) unclear. Possible explanations include impaired immune function, glutamine deficiency and insulin resistance (Takala et al 1999).

While GH treatment may reduce protein loss one of the side effects associated with GH therapy is insulin resistance which worsens the glucose intolerance associated with severe stress (Sherwin et al 1983). In the European trials the blood glucose concentrations ($p<0.001$) and insulin requirements ($p<0.001$) were higher in the GH treated patients compared with the patients receiving placebo, but there were no differences between the survivors and non-survivors in the GH treated groups (Takala et al 1999). In our study GH treatment was given in combination with IGF-I therapy to overcome this potential complication of GH treatment. Anabolic doses of IGF-I have a tendency to cause hypoglycaemia (Clemmons et al 1992). In the present study the plasma glucose concentration increased in the patients when nutritional $\pm$ hormonal support was provided although this increase only reached significance for the TPN and TPNGLN+GH/IGF-I treated patients. C-peptide levels, a reflection of

endogenous insulin secretion, were higher in Study 1 in the critically ill patients compared to the elderly normals (Chapter 4: 0.48 ± 0.13 nmol l^{-1}) indicating increased insulin secretion and remained high following treatment with TPNGLN+GH/IGF-I. Insulin levels also tended to be higher in Study 1 in the patients compared to the elderly normals (11.9 ± 2.7 mU l^{-1}) but the increase following TPNGLN+GH/IGF-I was not significant.

The multi-centre European studies also differed from the present study in that the patients were given a variety of nutritional supports excluding glutamine supplementation. An increase in protein anabolism as a result of GH treatment may result in a decrease in glutamine availability, exacerbating the glutamine depletion seen in the critically ill (Hinds 1999). It is possible that decreased glutamine availability may have contributed to the increased morbidity and mortality in the multi-centre ICU trials however gluamine levels were not recorded (Takala et al 1999).

When GH treatment (24 IU day^{-1}, subcutaneous) was given to patients receiving TPN following gastrointestinal surgery the glutamine release from the forearm muscle, measured by the arterio-venous balance technique, was abolished (Mjaaland et al 1993). Muscle biopsy studies in post-operative patients have shown that GH treatment reduces the fall in muscle free glutamine concentrations normally seen following surgery (Hammarqvist et al 1992). Biolo and colleagues have evaluated the acute effects (24 hour) of GH on muscle glutamine metabolism in severely traumatised patients after 1-2 weeks in an ICU. A local infusion of GH into the femoral artery (0.1 μg kg min^{-1}) reduced glutamine release from leg muscle by ~60 % and decreased the rate of de novo glutamine synthesis (Biolo et al 1997 and 1999). These results suggest that GH administration may have a potentially harmful affect on glutamine availability in the critically ill by suppressing muscle glutamine production.

In a porcine model of surgical trauma both chronic (3 days) and acute (1 dose on the morning of surgery) GH pre-treatment increased intestinal glutamine uptake decreased hind limb (skeletal muscle) net glutamine release and tended to reduce net glutamine uptake in the liver (Mjaaland et al 1995). In the same model GH pre-treatment (3 days) combined with a post-operative exogenous glutamine infusion also increased intestinal

glutamine uptake and reduced peripheral (muscle) glutamine release, but switched hepatic glutamine exchange from uptake to release (Unneberg et al 1996). In a similar model both GH and IGF-I (given independently, without exogenous glutamine) increased whole body Ra_{gln}, intestinal uptake and hepatic release of glutamine in the early stages of sepsis (Balteskard et al 1998). These experiments suggest that in the early stages of trauma GH pre-treatment induces an equilibrium in glutamine metabolism as the increased hepatic glutamine production may compensate for any reduction in muscle glutamine production.

Since the protein anabolic actions of growth hormone are primarily mediated by IGF-I there is also interest in the use of this hormone in the treatment of catabolic patients (Clemmons et al 1992, Lieberman et al 1994, Goeters et al 1995). In healthy adults rendered catabolic by a hypocaloric diet, 6 days of IGF-I treatment attenuated the negative nitrogen balance (Clemmons et al 1992). A significant, but transient improvement in N balance was reported in cachetic patients with acquired immunodeficiency syndrome receiving a low dose IGF-I infusion, 4 $\mu g\ h^{-1}\ kg^{-1}$ for 12 hours for 10 days (Lieberman et al 1994). However, there was no effect on whole body protein metabolism measured by [1-^{13}C] leucine following 10 days of IGF-I treatment. In a recent study of patients following gastric surgery (receiving TPN), the anabolic actions of IGF-I treatment (80 $\mu g\ kg^{-1}$, subcutaneous injection, twice a day for 6 days) on protein metabolism could not be shown when assessed by nitrogen balance or 3-methyl histidine excretion (Goeters et al 1995).

In their study of IGF-I in calorically restricted healthy adults Clemmons and colleagues reported that the IGF-I treatment tended to cause hypoglycaemia despite the fact that insulin levels were suppressed 3 to 4 fold (Clemmons et al 1992). The potency ratio of insulin to IGF-I to achieve similar reductions in plasma glucose levels in humans is 1:13 (Guler et al 1987). Using this conversion factor the IGF-I dose given in our study is equivalent to 0.23 $mU\ kg^{-1}\ day^{-1}$ of insulin. Kupfer and colleagues reported the addition of GH to the IGF-I treatment attenuated the hypoglycaemia (Kupfer et al 1993). A possible explanation for this is that the tendency of GH to cause insulin resistance counteracts the hypoglycaemic effects of IGF-I. As IGF-I treatment counteracts GH induced glucose intolerance, combined GH/IGF-I treatment may be a more appropriate treatment than either hormone alone. Furthermore in calorically

restricted normal adults combined IGF-I/GH treatment was also shown to have a greater anabolic effect than either hormone alone (Clemmons et al 1992, Kupfer et al 1993). Combined GH/IGF-I treatment has also been demonstrated to additively increase anabolism in parenterally fed rats subjected to surgical stress (Lo et al 1995).

In the present studies although there was a marked increase in GH levels in Study 2, this increase was not significant, however the final GH injection was given at 12:00 hours on the previous day. As anticipated the total IGF-I levels were significantly increased in Study 2 in the TPNGLN+GH/IGF-I treated patients, however the availability of IGF-I depends on its interaction with the IGF binding proteins. It has been reported that several days of IGF-I treatment causes increased IGFBP-1 and decreased IGFBP-3 levels (Young et al 1992, Kupfer et al 1993, Goeters et al 1995). In the present study IGFBP-1 levels in the critically ill patients in Study 1 tended to be higher than those reported for the elderly normals in Chapter 4 (35 ± 4 ng ml^{-1}). IGFBP-1 is found at higher levels in conditions of stress and is regulated by insulin and glucose levels (Baxter 1999). The liver is the major source of IGFBP-1 and it has been suggested that increased levels of this binding protein indicates hepatic insulin insensitivity (Baxter 1999).

IGFBP-3 is the main carrier protein for IGF-I and its levels are regulated by growth hormone. In critically ill patients IGFBP-3 levels have been shown to be decreased due to increased levels of a circulating protease that acts specifically on IGFBP-3 (Timmins et al 1996). This protease-induced increase in IGF-I availability could mean increased IGF-I delivery to target tissues or increased delivery to sites for disposal (Ross and Wilmore 1996). In the present study IGFBP-3 levels were initially lower in the critically ill patients compared with the elderly normals (Chapter 4: 3254 ± 325 ng ml^{-1}). GH induces IGFBP-3, whereas administration of IGF-I causes a down regulation of GH release which in turn causes the down regulation of IGFBP-3. In the present study IGFBP-3 levels were increased by nutritional support with combined GH/IGF-I therapy to near normal levels. Kupfer and co-workers also reported increased IGFBP-3 and ALS levels following combined GH/IGF-I treatment in calorically restricted normals (Kupfer et al 1993).

The patients receiving TPNGLN and TPNGLN+GH/IGF-I were given isonitrogenous nutritional support and the changes in plasma amino acid profiles were similar in both groups. Many of the amino acids were restored to near normal levels. However the changes in amino acid levels did not reach the same degree of statistical significance in the patients receiving TPNGLN+GH/IGF-I with significant increases only in the glutamine and phenylalanine levels. This is possibly due to the smaller patient number in this group.

The patients for the present study were recruited in the ICU from patients in whom the clinical decision had been made to use TPN. Unfortunately the study was terminated due to the withdrawal of GH from use in ICUs following the increased mortality in the GH multi-centre trials (Takala et al 1999). ICU patients are very heterogeneous and some of the trends between studies, for example the changes in insulin and amino acid concentrations, may have reached significance if we had been able to study more patients. The results show combined GH and IGF-I treatment did not decrease glutamine production rate in these critically ill patients. The addition of combined GH/IGF-I therapy to nutritional support with glutamine supplementation did not affect the increase in whole body glutamine uptake or the restoration of plasma glutamine concentration suggesting that in the presence of glutamine supplementation, this hormone treatment did not have adverse effects on glutamine metabolism.

Chapter 8

FINAL DISCUSSION

Glutamine is considered to be a "conditionally essential" amino acid during critical illness as it appears the metabolic demands for glutamine exceed endogenous production resulting in a decrease in free glutamine concentrations. To date it has not been clearly established whether this represents a true deficiency or an alteration in glutamine homeostasis related to severe illness (Darmaun et al 1998). The present studies have used isotopic tracer studies to assist in understanding the mechanisms for the changes in glutamine metabolism. As current parenteral amino acid regimes do not contain glutamine, there is considerable experimental and clinical interest in the use of glutamine supplementation in critically ill patients (Wernerman and Hammarqvist 1999, Griffiths 1999, Van Acker et al 1999). The current study was designed to be consistent with the clinical practice in ICU at St Thomas' where TPN is started as soon as possible if enteral nutrition is considered impractical.

Skeletal muscle is quantitatively the most important source of glutamine in the human body (Griffiths 1999, Van Acker et al 1999). Almost half the admissions to a general ICU are of patients over 60 years old (Griffiths 1997), and this is reflected in the fact that only five of the 17 patients in the present studies were younger than 60 years of age. It is well recognised that LBM declines with age. It was surprising that in our study there was no difference in LBM between the young and elderly healthy volunteers. In elderly ICU patients, especially those suffering from extensive or prolonged disease, the supply of glutamine may become limited due to depleted free pools or reduced capacity to produce glutamine caused by decreased muscle mass (Soeters and Olde Damink 1996, Griffiths 1997). Inability to meet the metabolic demands for glutamine could have severe consequences for tissues which consume glutamine at high rates such as the gastrointestinal tract and the immune system (Van Acker et al 1997).

The studies described in this thesis show that despite the fall in plasma glutamine levels there was no difference in Ra_{gln} between the critically ill patients and the matched healthy controls. However the tracer technique we have used measures whole body Ra_{gln} which reflects the average whole body Ra_{gln} from all tissues. Any increased

glutamine release from skeletal muscle may have been off set by decreased release from other tissues such as the liver (Van Acker et al 1999). Future studies may benefit from combining these tracer techniques with organ balance measurements to provide more specific information about glutamine metabolism in specific tissues. In the present studies glutamine uptake was increased in the ICU patients when exogenous glutamine was provided. This increased uptake may be for use by tissues with a high metabolic demand for glutamine or to replenish the muscle free pools. Unfortunately for ethical reasons we were unable to take muscle biopsy samples from the patients to monitor the intracellular muscle glutamine levels.

The patients in the current study were studied within 24 hours of admission to the ICU; at this stage the fall in plasma glutamine concentrations may represent use of an available amino acid pool rather than a true glutamine deficiency. It could be argued that in previously well-nourished, healthy individuals the initial metabolic responses seen during the first hours or days following acute trauma or illness are adaptive changes and do not require nutritional intervention (Van den Berghe et al 1998). However, the last three decades have seen considerable technical and therapeutic advances in intensive care medicine which can keep severely ill patients alive for weeks and months (Griffiths et al 1995, Van den Berghe et al 1998). Although a nutritional intervention is unlikely to alter early mortality in ICU patients it may speed recovery and reduce the length of stay in the ICU (Jones et al 1999). It would be interesting to investigate glutamine metabolism in a group of longer stay ICU patients to compare with the patients from the current study. Griffiths and colleagues reported a reduction in 6 month mortality in ICU patients who were treated with TPN supplemented with glutamine compared to those receiving standard TPN (Griffiths et al 1997). The excess deaths in the group not receiving glutamine supplementation occurred after 21 days which supports the argument that with progressive skeletal muscle loss endogenous glutamine supply becomes critical to survival (Griffiths 1997).

Supplementation of parenteral nutrition with glutamine could be considered correction of a nutritional deficiency rather than additional treatment however enteral nutrition does contain glutamine (Jones et al 1999, Wernerman and Hammarqvist 1999). Current ICU practice is to introduce enteral nutrition as soon as possible and only introduce parenteral nutrition if enteral nutrition fails or is impractical. When enteral

nutrition in ICU patients was supplemented with additional glutamine there was no difference in mortality rates between the patients receiving standard or supplemented nutrition, but there was a significant reduction in hospital patient costs in the glutamine treated patients (30 % per survivor) (Jones et al 1999). The difference in hospital costs was partly due to shorter ICU stays, decreased need for supplemental parenteral feeding, and shorter post-ICU stays in the glutamine supplemented patients. It would therefore be of interest to investigate glutamine metabolism using isotopic tracers in patients receiving enteral nutrition.

It has been estimated that ICU patients lose 2 % of skeletal muscle per day, despite nutritional support (Griffiths 1997). This continued loss has led to interest in the potential of GH to preserve muscle mass in these patients. However, since the first major trial in ICU patients revealed a doubling of mortality in GH-treated patients compared with placebo treated patients, further use of GH in ICUs will not be permitted for some time (Takala et al 1999). The precise mechanism(s) for this finding remain speculative, but it is important to determine why GH treatment had such an effect.

Although the study described in this thesis is in only a small group of ICU patients the administration of GH/IGF-I with glutamine supplementation did not appear to have deleterious effects. The decision to include glutamine supplementation in the GH/IGF-I treated patients was made before the results of the multi-centre ICU trials became apparent. As GH is known to be protein anabolic we were concerned that GH treatment could exacerbate the glutamine depletion in these catabolic patients. Although it would have been interesting to study the effects of the combined hormonal treatment on patients receiving standard TPN, we felt it would be unethical to give this treatment without glutamine supplementation. In addition, unlike the multi-centre trial, we gave combined GH/IGF-I treatment to overcome the potential hyperglycaemic complications associated with GH therapy

The decision by the pharmaceutical industry to withdraw GH from use in the ICU is understandable, but may be premature. There have been numerous small studies investigating the use of GH treatment in catabolic patients where GH was shown to be either beneficial or not harmful. The differences in outcome between the previous GH

studies and the multi-centre trial may reflect differences in the patient populations and their respective neuroendocrine states (Van den Berghe 1999, Demling 1999). Many of the earlier studies involved non-ICU patients such as patients with AIDS wasting or patients recovering from burns (including children) or patients in the first days following elective surgery, trauma or sepsis. In the major multi-centre trial patients were included 5-7 days after life threatening insult if it was anticipated that their intensive care dependency would be prolonged. Furthermore the high doses of GH used (16-24 IU day^{-1}) in the multi-centre trial may have been a contributing factor to the negative outcome (Isaksson 1999). High GH doses are used in ICU studies as critically ill patients are considered to be GH resistant. This may be true in acute critical illness but more recent data published while the GH trial was ongoing suggests that in prolonged critical illness patients are not necessarily GH resistant (Van den Berghe 1999). It is possible that GH treatment may be beneficial at a later stage in the patients' recovery, with lower doses adjusted individually to achieve a defined degree of clinical/metabolic response (Isaksson 1999).

In conclusion, the findings of this study support the use of glutamine supplementation with parenteral nutrition. The protein turnover studies performed in conjunction with the studies presented in this thesis indicated that GH/IGF-I treatment improved whole body protein balance (Carroll et al 1999). Together with the lack of effect on glutamine metabolism these findings suggest that GH/IGF-I treatment may be of benefit to critically ill patients when glutamine supplementation is provided. It is important that more clinical research is undertaken to investigate further the roles of both glutamine supplementation and GH/IGF-I treatment in ICU patients.

References

Abumard, N. N., D. Rabin, M. P. Diamond, and W. W. Lacy. Use of a heated superficial hand vein as an alternative site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. *Metabolism* 30: 936-940, 1981.

Ahmed, A., D. L. Maxwell, P. M. Taylor, and M. J. Rennie. Glutamine transport in human skeletal muscle. *Am. J. Physiol.* 264 (*Endocrinol. Metab.* 27): E993-E1000, 1993.

Albers, S., J. Wernerman, P. Stehle, E. Vinnars, and P. Fürst. Availability of amino acids supplied intravenously in healthy man as synthetic dipeptides: kinetic evaluation of L-alanyl-L-glutamine and glycyl-L-tryosine. *Clin. Sci.* 75: 463-468, 1988.

Anderson, L. W., D. W. Zaharevitz, and J. M. Strong. Glutamine and glutamate: automated quantification and isotopic enrichments by gas chromatography/mass spectrometry. *Anal. Biochem.* 163: 358-368, 1987.

Ardawi, M. S. M., and E. A. Newsholme. Metabolism in lymphocytes and its importance in the immune response. *Essays Biochem.* 21: 1-44, 1985.

Ardawi, M. S. M., M. F. Majzoub, and E. A. Newsholme. Effect of glucocorticoid treatment on glucose and glutamine metabolism by the small intestine of the rat. *Clin. Sci.* 75: 93-100, 1988.

Askanazi, J., Y. A. Carpentier, C. B. Michelsen, D. H. Elwyn, P. Fürst, L. R. Kantrowitz, F. E. Gump, and M. J. Kinney. Muscle and plasma amino acids following injury: influence of intercurrent infection. *Ann. Surg.* 192: 78-85, 1980.

Balteskard, L., K. Unneberg, M. Mjaaland, T. G. Jenssen, and A. Revhaug. Growth hormone and insulinlike growth factor 1 promote intestinal uptake and hepatic release of glutamine in sepsis. *Ann. Surg.* 228: 131-139, 1998.

Baumann, G., K. D. Amburn, and T. A. Buchanan. The effect of circulating growth hormone-binding protein on metabolic clearance, distribution and degradation of human growth hormone. *J. Clin. Endocrinol. Metab.* 64: 657-660, 1987.

Baumann, G., K. Amburn, and M. A. Shaw. The circulating growth hormone (GH)-binding protein complex: a major of constituent of plasma GH in man. *Endocrinology* 122: 976-984, 1988.

Baumann, G. Growth hormone binding proteins. *Endocrine Updates-Growth Hormone.* ed. B.-A. Bengtsson, Kluwer Academic Publishers, p37-57, 1999.

Baxter, R. C. The insulin-like growth factor (IGF)-IGF-binding protein axis in critical illness. *GH & IGF Res.* 9: 67-69, 1999.

Bergström, J., P. Fürst, L.-O. Norée, and E. Vinnars. Intracellular free amino acid concentration in human muscle tissue. *J. App. Physiol.* 36: 693-697, 1974.

Berman, R. S., L. E. Harrison, D. B. Pearlstone, M. Burt, and M. F. Brennan. Growth hormone, alone and in combination with insulin, increases whole body and skeletal muscle protein kinetics in cancer patients after surgery. *Ann. Surg.* 229: 1-10, 1999.

Biolo, G., F. Iscra, G. Toigo, B. Ciocchi, R. Situlin, A. Gullo, and G. Guarnie. Effects of growth hormone administration on skeletal muscle glutamine metabolism in severely traumatized patients: preliminary report. *Clin. Nutr.* 16: 89-91, 1997.

Biolo, G., F. Iscra, G. Toigo, B. Ciocchi, L. Cocchia, A. Gullo, and G. Guarnie. Growth hormone decreases glutamine production while stimulates muscle protein synthesis in severely traumatized patients. 5th International symposium on amino acid/protein metabolism in health and disease. Perugia, Italy, September 1999.

Blomqvist, B. I., F. Hammerqvist, A. Von der Decken, and J. Wernerman. Glutamine and ketoglutarate prevent the decrease in muscle free glutamine concentration and influence protein synthesis after hip replacement. *Metabolism* 44: 1215-1222, 1995.

Bode, B, B. K. Tamarappoo, M. Mailliard, and M. S. Kilberg. Characteristics and regulation of hepatic glutamine transport. *J.P.E.N.* 14: 51S-55S, 1990.

Bode, B. P., D. L. Kaminski, W. W. Souba, and A. P. Li. Glutamine transport in isolated human hepatocytes and transformed liver cells. *Hepatology* 21: 511-520, 1995.

Brillon, D. J., B. Zheng, R. G. Campbell, and D. E. Matthews. Effect of cortisol on energy expenditure and amino acid metabolism in humans. *Am. J. Physiol.* 268 (*Endocrinol. Metab.* 31): E501-E513, 1995.

Bussolotto, M., A. Ceccin, G. Sergi, V. Giantin, P Beninca, and G. Enzi. Assessment of body compostion in elderly: accuracy of bioelectrical impedance analysis. *Gerontology* 45: 39-43, 1999.

Cao, Y., Z. Feng, A. Hoos, and V. S. Klimberg. Glutamine enhances gut glutathione production. *J.P.E.N.* 22: 224-227, 1998.

Carbonnel, F., B. Messing, D. Darmaun, A. Rimbert, M. Rongier, O. Rigal, J. Koziet, F. Thuillier, and J. F. Desjeux. Energy and protein metabolism in malnutrition due to nonneoplastic gastrointestinal diseases. *Metabolism* 44: 1110-1115, 1995.

Carli, F., J. D. Webster, and D. Halliday. Growth hormone modulates amino acid oxidation in the surgical patient: leucine kinetics during the fasted and fed state using moderate nitorgenous and caloric diet and recombinant human growth hormone. *Metabolism* 46: 23-28, 1997.

Carroll, P. V., N. Jackson, P. Sönksen, D. Treacher, D. Russell-Jones, and A. M. Umpleby. Combined GH/IGF-I treatment results in net protein anabolism in critically ill patients receiving TPN supplemented with glutamine. 5th International symposium on amino acid/protein metabolism in health and disease. Perugia, Italy, September 1999.

Cerra, F. B., M. R. Benitez, G. L. Blackburn, R. S. Irwin, K. Jeejeebhoy, D. P. Katz, S. K. Pingleton, J. Pomposelli, J. L. Rombeau, E. Shronts, R. R. Wolfe and G. P. Zaloga. Applied nutrition in ICU patients: a consensus statement of the american college of chest physicians. *Chest* 111: 769-778, 1997.

Chaves Das Neves, H. J., and A. M. P. Vasconcelos. Capillary gas chromatography of amino acids including, asparagine and glutamine: senstive gas chromatgraphic-mass spectrometric and selected ion monitoring gas chromatgraphic-mass spectrometric detection of the N,O(S)-*tert*-butyldimethylsilyl derivatives. *J. Chrom.* 392: 249-258, 1987.

Chopra, I. J., T.S. Huang, A. Beredo, D. H. Solomon, G. N. Chua Teco, and J. F. Mead. Evidence for an inhibitor of extrathyroidal conversion of thyroxine to 3,5,3'-triiodothyronine in sera of patients with non-thyroidal illness. *J. Clin. Endocrinol. Metab.* 60: 660-672, 1985.

Clemmons, D. R., A. Smith-Banks, and L. E. Underwood. Reversal of diet-induced catabolism by infusion of recombinant insulin-like growth factor-I (IGF-I) in humans. *J. Clin. Endocrinol. Metab.* 75: 234-238, 1992.

Copeland, K. C. and K. S. Nair. Acute growth hormone effects on amino acid and lipid metabolism. *J. Clin. Endocrinol. Metab.* 78: 1010-1047, 1994.

Cullen, D. J., J. M. Civetta, B. A. Briggs, and L. C. Ferrara. Therapeutic intervention scoring system: a method for quantitative comparison of patient care. *Crit. Care Med.* 2: 57-60, 1974.

Cuneo, R. C., F. Salomon, C. M. Wiles, R. Hesp, and P. H. Sönksen. Growth hormone treatment in growth hormone-deficient adults. I. effects on muscle mass and strength. *J. Appl. Physiol.* 70: 688-694, 1991.

Darmaun, D., M. J. Manary, and D. E. Matthews. A method for measuring both glutamine and glutamate levels and stable isotopic enrichments. *Anal. Biochem.* 147: 92-102, 1985.

Darmaun, D., D. E. Matthews, and D. M. Bier. Glutamine and glutamate kinetics in humans. *Am. J. Physiol.* 251 (*Endocrinol. Metab.* 14): E117-E126, 1986.

Darmaun, D., D. E. Matthews, and D. M. Bier. Physiological hypercortisolemia increases proteolysis, glutamine, and alanine production. *Am. J. Physiol.* 255 (*Endocrinol. Metab.* 18): E366-E373, 1988.

Darmaun, D., and P. Dechelotte. Role of leucine as a precursor of glutamine alpha-amino nitrogen in vivo in humans. *Am. J. Physiol.* 260 (*Endocrinol. Metab.* 23): E326-E329, 1991.

Darmaun, D., B. Messing, B. Just, M. Rongier, and J.-F. Desjeux. Glutamine metabolism after small intestinal resection in humans. *Metabolism* 40: 42-44, 1991a.

Darmaun, D., M. Rongier, J. Koziet, and J.-J. Robert. Glutamine nitrogen kinetics in insulin-dependent diabetic humans. *Am. J. Physiol.* 261 (*Endocrinol. Metab.* 24): E713-E718, 1991b.

Darmaun, D., B. Just, B. Messing, M. Rongier, F. Thuillier, J. Koziet, and E. Grasset. Glutamine metabolism in healthy adult men: response to enteral and intravenous feeding. *Am. J. Clin. Nutr.* 59:1395-1402, 1994.

Darmaun, D., S. Welch, A. Rini, B. K. Sager, A. Altomare, and M. W. Haymond. Phenylbutyrate-induced glutamine depletion in humans: effect on leucine metabolism. *Am. J. Physiol.* 274 (*Endocrinol. Metab.* 37): E801-E807, 1998.

Demling, R. Growth hormone therapy in critically ill patients. *N.E.J.M.* 341: 837-839, 1999.

Docter, R., E. P. Kenning, M. De Jong, and G. Henneman. The sick euthyriod syndrome changes in thyroid hormone serum parameters and hormone metabolism. *Clin. Endocrinol.* 39: 499-518, 1993.

Douglas, R. G., D. A. Humberstone, A. Haystead, and J. H. F. Shaw. Metabolic effects of recombinant human growth hormone: isotopic studies in the postabsorptive state and during total parenteral nutrition. *Br. J. Surg.* 77: 785-790, 1990.

Enzi, G., G. Sergi, M. Bussolotto, A. Ceccon, V. Giantin, and P. Benincà. Methods for quantification of body composition, with particular reference to lean body mass. *Amino acid and protein metabolism in health and disease.* eds. P. Tessari, P.B. Soeters, G. Pittoni and A. Tienzo, Smith-Gordon, p5-11, 1997.

Essen, P., J. Wernerman, T. Sonnenfeld, S. Thunell, and E. Vinnars. Free amino acids in plasma and muscle during 24 hours post-operatively - a descriptive study. *Clin. Physiol.* 12: 163-77, 1992.

Evans, N. J. The role of total parenteral nutrition in critical illness: guidelines and recommendations. *A.A.C.N.* 5: 476-484, 1994.

Felig, P., J. Wahren, I. Karl, E. Cerasi, R. Luft, and D. M. Kipnis. Glutamine and glutamate metabolism in normal and diabetic subjects. *Diabetes* 22: 573-576, 1973.

Ford, G. C., K. N. Cheng, and D. Halliday. Analysis of (1-^{13}C) leucine and (^{13}C) KIC in plasma by capillary gas chromatography/mass spectrometry in protein turnover studies. *Biomed. Mass Spec.* 12: 432-436, 1985.

Fryburg, D. A., R. A. Gelfand, and E. J. Barrett. Growth hormone acutely stimulates forearm muscle protein synthesis in normal humans. *Am. J. Physiol.* 260 (*Endocrinol. Metab.* 23): E499-504, 1991.

Fryburg, D. A., and E. J. Barrett. Insulin, growth hormone and IGF-I regulation of protein metabolism. *Diab. Rev.* 3: 93-112, 1995.

Fürst, P., S. Albers, and P. Stehle. Availabilty of glutamine supplied intravenously as alanylglutamine. *Metabolism* 38: 67S-72S, 1989.

Gamrin, L., P. Essén, A. M. Forsberg, E. Hultman, and J. Wernerman. A descriptive study of skeletal muscle metabolism in critically ill patients: free amino acids, energy-rich phosphates, protein, nucleic acids, fat, water and electrolytes. *Crit. Care Med.* 24: 575-583, 1996.

Garlick, P. J., and I. Grant. Amino acid infusion increases the sensitivity of muscle protein synthesis in vivo to insulin. Effect of branched-chain amino acids. *Biochem. J.* 254: 579-584, 1988.

Gelfand, R. A., and E. J. Barrett. Effect of physiologic hyperinsulinaemia on skeletal muscle protein synthesis and breakdown in man. *J. Clin. Invest.* 80: 1-6, 1987.

Goeters, C., N. Mertes, J. Tacke, U. Bolder, M. Kuhmann, P. Lawin, and D. Löhlein. Repeated administration of recombinant human insulin-like growth factor-I in patients after gastric surgery effect on metabolic and hormonal patterns. *Ann. Surg.* 222: 646-653, 1995.

Golden, M. H. N., P. Jahoor, and A. A. Jackson. Glutamine production rate and its contribution to urinary ammonia in normal man. *Clin. Sci.* 62: 299-305, 1982.

Gore, D. C., and F. Jahoor. Glutamine kinetics in burn patients, comparison with hormonally induced stress in volunteers. *Arch. Surg.* 129: 1318-1323, 1994.

Griffiths, R. D. Outcome of critically ill patients after supplementation with glutamine. *Nutrition* 13: 752-754, 1997.

Griffiths, R. D. Glutamine: establishing clinical indications. *Cur. Op. Clin. Nutr. Metab. Care* 2: 177-182, 1999.

Griffiths, R. D., C. Jones, and T. E. A. Palmer. Outcome of nutrition therapies in the intensive care unit. *Nutrition* 11: 224S-228S, 1995.

Griffiths, R. D., C. Jones, and T. E. A. Palmer. Six-month outcome of critically ill patients given glutamine-supplemented parenteral nutrition. *Nutrition* 13: 295-302, 1997.

Guler, H.-P., J. Zapf, and E. R. Froesch. Short-term metabolic effects of recombinant insulin-like growth factor in healthy adults. *N.E.J.M.* 317: 137-140, 1987.

Hammarqvist, F., J. Wernerman, A. Rustom, A. Von der Decken, and E. Vinnars. Addition of glutamine to total parenteral nutrition after elective abdominal surgery spares free glutamine in muscle, counteracts the fall in muscle protein synthesis, and improves nitrogen balance. *Ann. Surg.* 209: 455-461, 1989.

Hammarqvist, F., J. Wernerman, A. Von der Decken, and E. Vinnars. Alanyl-glutamine counteracts the depletion of free glutamine and the postoperative decline in protein synthesis in skeletal muscle. *Ann. Surg.* 212: 637-644, 1990.

Hammarqvist, F., C. Stromberg, A. Von der Decken, E. Vinnars, and J. Wernerman. Biosynthetic human growth hormone preserves both muscle protein synthesis and the decrease in muscle-free glutamine, and improves whole-body nitrogen economy after operation. *Ann. Surg.* 216: 184-191, 1992.

Hankard, R. G., D. Darmaun, B. K. Sager, D. D'Amore, W. R. Parsons and M. Haymond. Response of glutamine metabolism to exogenous glutamine in humans. *Am. J. Physiol.* 269 (*Endocrinol. Metab.* 32): E663-E670, 1995.

Hankard, R. G., M. W. Haymond, and D. Darmaun. Effect of glutamine on leucine metabolism in humans. *Am. J. Physiol.* 271 (*Endocrinol. Metab.* 34): E748-E754, 1996.

Hankard, R.G., M. W. Haymond, and D. Darmaun. Role of glutamine as a glucose precursor in fasting humans. *Diabetes* 46: 1535-1541, 1997.

Hankard, R. G., D. Hammond, M. W. Haymond, and D. Darmaun. Oral glutamine slows down whole body protein breakdown in duchenne muscular dystrophy. *Pediatr. Res.* 43: 222-226, 1998.

Hardy, G., D. Wiggins, B. McElroy, and G. R. Thompson. Formulation of a glutamine-containing total parenteral nutrition mixture for clinical use. *Proc. Nutr. Soc.* 51: 136A, 1992.

Hardy, G., S. J. Bevan, B. McElroy, T. E. A. Plamer, R. D. Griffiths, and C. Braidwood. Stability of glutamine in parenteral feeding solutions. *Lancet* 432: 186, 1993.

Harward, T. R., D. Coe, W. W. Souba, N. Klingman, and J. M. Seeger. Glutamine preserves gut glutathione levels during intestinal ischemia/reperfusion. *J. Surg. Res.* 56: 315-355, 1994.

Häussinger, D. Liver glutamine metabolism. *J.P.E.N.* 14: 56S-62S, 1990.

Hinds, C. J. Administration of growth hormone to catabolic patients. *GH & IGF-I Res.* 9: 71-75, 1999.

Holmes, S. J., G. Economou, R. W. Whitehouse, J. E. Adams, and S. M. Shalet. Reduced bone mineral density in patients with adult onset growth hormone deficiency. *J. Clin. Endocrinol. Metab.* 78: 669-674, 1994.

Hong, R. W., J. D. Rounds, W. S. Helton, M. K. Robinson, and D. W. Wilmore. Glutamine preserves liver glutathione after lethal hepatic injury. *Ann. Surg.* 215: 114-119, 1992.

Hübl, W., W. Druml, K. Langer, and H. Lochs. Influence of molecular structure and plasma hydrolysis on the metabolism of glutamine-containing dipeptides in humans. *Metabolism* 38: 59S-62S, 1989.

Hundal, H. S., M. J. Rennie, and P. W. Watt. Characteristics of L-glutamine transport in perfused rat skeletal muscle. *J. Physiol.* 393: 283-305, 1987.

Isaksson, O. G. P. Growth hormone treatment in critical illness -a critical appraisal. *GH & IGF Res.* 9: 83-84, 1999.

Jacobs, D. O. Use of bioelectrical impedance analysis measurements in the clinical management of critical illness. *Am. J. Clin. Nutr.* 64: 498S-502S, 1996.

Jeevanandam, M., N. J. Holaday, and S. R. Petersen. Integrated nutritional, hormonal, and metabolic effects of recombinant human growth hormone (rhGH) supplementation in trauma patients. *Nutrition* 12: 777-787, 1996.

Jenkins, C. R., and R. J. M. Ross. The endocrinology of the critically ill. *Cur. Op. Endocrinol. Diabetes* 3: 138-145, 1996.

Jepson, M. M., P. C. Bates, P. Broadbent, J. M. Pell, and D. J. Millward. Relationship between glutamine concentration and protein synthetesis in rat skeletal muscle. *Am. J. Physiol.* 255 (*Endocrinol. Metab.* 18): E166-E172, 1988.

Jones, C., T. E. A. Palmer, and R. D. Griffiths. Randomized clinical outcome study of critically ill patients given glutamine-supplemented enteral nutrition. *Nutrition* 15: 108-115, 1999.

Jurney, T. H., J. S. Lindberg, J. M. Lamiell, and C. E. Wade. Spectrum of serum cortisol response to ACTH in ICU patients: correlation with degree of illness and mortality. *Chest* 92: 292-295, 1987.

Kapadia, C. R., M. R. Colpoys, Z.-M. Jiang, D. J. Johnson, R. J. Smith, and D. Wilmore. Maintenance of intracellular glutamine during standard surgical trauma. *J.P.E.N.* 9: 583-589, 1985.

Kaptein, E. M., D. A. Grieb, C. Spencer, W.S. Wheeler, and J. T. Nicoloff. Thyroxine metabolism in the low thyroxine state of critical non-thyroidal illnesses. *J. Clin. Endocrinol. Metab.* 53: 764-771, 1981.

Kilberg, M. S., M. E. Handlogten, and H. N. Christensen. Charatceristics of an amino acid transport system in rat liver for glutamine, asparagine, histidine and closely related analogs. *J. Biol. Chem.* 255: 4011-4019, 1980.

Kinney, J. M. and D. H. Elwyn. Protein metabolism and injury. *Ann. Rev. Nutr.* 3: 433-466, 1983.

Knaus, W. A., E. A. Draper, D. P. Wagner, and J. E. Zimmerman. APACHE II: a severity of disease classification system. *Crit. Care Med.* 13: 818-829, 1985.

Krebs, H. Special lecture: glutamine metabolism in the animal body. *Glutamine metabolism, enzymology, and regulation.* ed. J. Mora and R. Palacios, New York Academic Press, p319-329, 1980.

Kreider, M. E., M. Stumvoll, C. Meyer, D. Overkamp, S. Welle, and J. Gerich. Steady-state and non-steady-state measurements of plasma glutamine turnover in humans. *Am. J. Physiol.* 272 (*Endocrinol. Metab.* 35): E621-E627, 1997.

Kupfer, S. R., L. E. Underwood, R. C. Baxter, and D. R. Clemmons. Enhancement of the anabolic effects of growth hormone and insulin-like growth factor I by use of both agents simultaneously. *J. Clin. Invest.* 91: 391-396, 1993.

Lacey, J. M., and D. W. Wilmore. Is glutamine a conditionally essential amino acid ? *Nutrition Reviews* 48: 297-309, 1990.

Lieberman, S. A., G. E. Butterfield, D. Harrison, and A. R. Hoffman. Anabolic effects of recombinant insulin-like growth factor-I in cachectic patients with the acquired immunodeficiency syndrome. *J. Clin. Endocrinol. Metab.* 78: 404-410, 1994.

Lo, H.-C., P. S. Hinton, C. A. Peterson, and D. M. Ney. Simultaneous treatment with IGF-I and GH additively increases anabolism in parenterally fed rats. *Am. J. Physiol.* 269 (*Endocrinol. Metab.* 32): E368-E376, 1995.

Long, C. L., K. M. Nelson, D. B. DiRenzo, J. K. Weis, R. D. Stahl, T. D. Broussard, W. L. Theus, J. A. Clark, T. W. Pinson, J. W. Geiger, H. L. Laws, W. S. Blakemore, and R. P. Carraway. Glutamine supplementation of enteral nutrition: impact on whole body protein kinetics and glucose metabolism in critically ill patients. *J.P.E.N.* 19: 470-476, 1995.

Lukaski, H. C., W. W. Bolonchunk, C. B. Hall, and W. A. Siders. Validation of tetrapolar bioelectrical impedance method to assess human body composition. *J. Appl. Physiol.* 60: 1327-1332, 1986.

MacBurney, M., L. S. Young, T. R. Zeigler, and D. W. Wilmore. A cost-evalutation of glutamine supplemented parenteral nutrition in adult bone marrow transplant patients. *J. Am. Diet. Assoc.* 94: 1263-1266, 1994.

MacLennan, P. A., R. A. Brown, and M. J. Rennie. A positive relationship between protein synthetic rate and intracellular glutamine concentration in perfused rat skeletal muscle. *F.E.B.S.* 215: 187-191, 1987.

MacLennan, P. A., K. Smith, B. Weryk, P. W. Watt, and M. J. Rennie. Inhibition of protein breakdown by glutamine in perfused rat skeletal muscle. *F.E.B.S.* 237: 133-136, 1988.

Manning, E. M. C., and A. Shenkin. Nutritional assessment in the critically ill. *Crit. Care Clinics* 11: 603-634, 1995.

Manson, J. M., and D. W. Wilmore. Positive nitrogen balance with human growth hormone and hypocaloric intravenous feeding. *Surgery* 100: 188-196, 1986.

Marliss, E. B., T. T. Aoki, T. Pozefsky, A. S. Most, and G. F. Cahill. Muscle and splanchnic glutamine and glutamate metabolism in postabsorptive and starved man. *J. Clin. Invest.* 50: 814-817, 1971.

Matthews, D. E., K. J. Motil, D. K. Rohrbaugh, J. F. Burke, V. R. Young, and D. M. Bier. Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-^{13}C] leucine. *Am. J. Physiol.* 238 (*Endocrinol. Metab.* 1): E473-E479, 1980.

Matthews, D. E., H. P. Schwartz, R. D. Yang, K. J. Motil, V. R. Young, and D. M. Bier. Relationship of plasma leucine and α-ketoisocaproate during a L-[1-^{13}C] leucine infusion in man: a method for measuring human intracellular leucine tracer enrichment. *Metabolism* 31: 1105-1112, 1982.

Matthews, D. E., G. Pesola, and R. G. Campbell. Effect of epinephrine on amino acid and energy metabolism in humans. *Am. J. Physiol.* 258 (*Endocrinol. Metab.* 21): E948-E956, 1990.

Matthews, D. E., and R. G. Campbell. The effect of dietary protein intake on glutamine and glutamate nitrogen metabolism in humans. *Am. J. Clin. Nutr.* 55: 963-700, 1992.

Matthews, D. E., M. A. Marano, and R. G. Campbell. Splanchnic bed utilization of glutamine and glutamic acid in humans. *Am. J. Physiol.* 264 (*Endocrinol. Metab.* 27): E848-E854, 1993.

McGivan, J. D., and M. Pastor-Anglada Regulatory and molecular aspects of mammalian amino acid transport. *Biochem. J.* 299: 321-334, 1994.

McGivan, J. D. Mammalian amino acid transporters and their regulation. *Biochem. Soc. Trans.* 24: 837-838-334, 1996.

Meister, A. Enzymology of glutamine. *Glutamine metabolism in mammalian tissues.* ed. D. Häussinger and H. Sies, Springer-Verlag, p3-15, 1984.

Meakins, J. L., and J. C. Marshall. The gastrointestinal tract: the motor of multiple organ failure. *Arch. Surg.* 121: 197-201, 1986.

Messing, B., S. L. Dutra, F. Thuillier, D. Darmaun, and J. F. Desjeux. Whole-body protein metabolism assessed by leucine and glutamine kinetics in adult patients with active celiac disease. *Metabolism* 47: 1429-1433, 1998.

Miell, J. P., A. M. Taylor, J. Jones, C. R. Buchanan, J. Rennie, R. Sherwood, R Leicester, and R. J. M. Ross. Administration of human recombinant insulin-like growth factor-I to patients following major gastrointestinal surgery. *Clin. Endocrinol.* 37: 542-551, 1992.

Mjaaland, M., K. Unneberg, J. Larsson, L. Nilsson, and A. Revhaug. Growth hormone after abdominal surgery attenuated forearm glutamine, alanine, 3-methylhistidine, and total amino acid efflux in patients receiving total parenteral nutrition. *Ann. Surg.* 217: 413-422, 1993.

Mjaaland, M., K. Unneberg, K. Jenssen, and A. Revhaug. Experimental study to show that growth hormone treatment before trauma increases glutamine uptake in the intestinal tract. *Br. J. Surg.* 82: 1076-1079, 1995.

Munck, A., P. Guyre, and N. Holbrook. Physiolgical functions of glucocorticoids during stress and their relation to pharmacological actions. *Endocr. Rev.* 5: 25-44, 1984.

Muhlbacher, F., C. R. Kapadia, M. F. Colpoys, R. J. Smith, and D. W. Wimore. Effects of glucocorticoids on glutamine metabolism in skeletal muscle. *Am. J. Physiol.* 247 (*Endocrinol. Metab.* 10): E75-E83, 1984.

Neptune, E. M. Respiration and oxidation of various substrates by ileum in vitro. *Am. J. Physiol.* 209: 329-333, 1965.

Neu, J., V. Shenoy, and R. Chakrabarti. Glutamine nutrition and metabolism: where do we go from here ? *FASEB J.* 10: 829-837, 1996.

Nissm, I., M. Yudkoff, and A. Lapidot. Simultaneous determination of [2-^{15}N]- and [5-^{15}N]glutamine with gas chromatography-mass spectroscopy: applications to nitrogen metabolic studies. *Anal. Biochem.* 143: 14-20, 1984.

Nurjhan, N., A. Bucci, G. Perriello, M. Stumvoll, G. Dailey, D. M. Bier, I. Toft, T. G. Jenssen, and J. E. Gerich. Glutamine: a major gluconeogenic precursor and vehicle for interorgan carbon transport in man. *J. Clin. Invest.* 95: 272-277, 1995.

Olde Damink, S. W., I. de Blaauw, N. E. Deutz, and P. B. Soeters. Effects in vivo of decreased plasma and intracellular muscle glutamine concentration on whole-body and hindquarter protein kinetics in rats. *Clin. Sci.* 96: 639-646, 1999.

Owen, E. E., and R. R. Robinson. Amino acid extraction and ammonia metabolism by the human kidney during prolonged administration of ammonium chloride. *J. Clin. Invest.* 42: 263-276, 1963.

Pacitti, A. J., Y. Inoue, D. A. Plumley, E. M. Copeland and W.W. Souba. Growth hormone regulates amino acid transport in human and rat liver. *Ann. Surg.* 216: 353-361, 1992.

Palacín, M., R. Estévez, J. Bertran, and A. Zorzano. Molecular biology of mammalian plasma membrane amino acid transporters. *Physiol. Rev.* 78: 969-1054, 1998.

Palmer, T. E., R. D. Griffiths, and C. Jones. Effect of parenteral L-glutamine on muscle in the very severely ill. *Nutrition* 12: 316-320, 1996.

Parry-Billings, M., J. Evans, P. C. Calder, and E. A. Newsholme. Does glutamine contribute to immunosupression after major burns ? *Lancet* 336: 523-525, 1990.

Perriello, G., R. Jorde, N. Nurjhan, M. Stumvoll, G. Dailey, T. Jenssen, D. M. Bier, and J. E. Gerich. Estimation of the glucose-alanine-lactate-glutamine cycles in

postabsorptive man: role of skeletal muscle. *Am. J. Physiol.* 269 (*Endocrinol. Metab.* 32): E443-E450, 1995.

Perrot, D., A. Bonneton, H. Dechaud, J. Motin, and M. Pugeat. Hypercortisolism in septic shock is not supressible by dexamethasone infusion. *Crit. Care Med.* 21: 396-401, 1993.

Petersson, B., E. Vinnars, S. O. Waller, and J. Wernerman. Long-term changes in muscle free amino acid levels after elective abdominal surgery. *Br. J. Surg.* 79: 212-216, 1992.

Petersson, B., S. O. Waller, E. Vinnars, and J. Wernerman. Long term effect of glycyl-glutamine after elective surgery on free amino acids in muscle. *J.P.E.N.* 18: 320-325, 1994.

Pittoni, G., G. Gallioli, M. Zanello, E. Ghigo, and G. Davià. Anabolic hormone profiles in severe catabolism. *Amino acid and protein metabolism in health and disease*. eds. P. Tessari, P.B. Soeters, G. Pittoni and A. Tienzo, Smith-Gordon, p161-186, 1997.

Ponting, G. A., H. C. Ward, D. Halliday, and A. J. W. Sim. Protein and energy metabolism with biosynthetic human growth hormone in patients on full intravenous nutritional support. *J.P.E.N.* 14: 437-441, 1990.

Powell-Tuck, J. Glutamine, parenteral feeding, and intestinal nutrition. *Lancet* 342: 451-452, 1993.

Reincke, M., B. Allolio, G. Wurth, and W. Winkelmann. The hypothalamic-pituitary-adrenal axis in critical illness: response to dexamethasone and cortiotrophin-releasing hormone. *J. Clin. Endocrinol. Metab.* 77: 151-156, 1993.

Robert, J.-J., D. M. Bier, X. H. Zhao, D. E. Matthews, and V. R. Young. Glucose and insulin effects on de novo amino acid synthesis in young men: studies with stable isotope labeled alanine, glycine, leucine, and lysine. *Metabolism* 31: 1210-1218, 1982.

Rolih, C. A., and K. P. Ober. The endocrine response to critical illness. *Med. Clin. North Am.* 79: 211-224, 1995.

Ross, R., J. Miell, E. Freeman, J. Jones, D. Matthews, M. Preece, and C. Buchanan. Critically ill patients have high basal growth hormone levels with attenuated oscillatory activity associated with low levels of insulin-like growth factor-I. *Clin. Endocrinol.* 35: 47-54, 1991a.

Ross, R. J. M., J. P. Miell, J. M. P. Holly, H. Maheshwari, M. Norman, A. F. Abdulla, and C. R. Buchanan. Levels of GH binding activity, IGFBP-I, insulin, blood glucose and cortisol in intensive care patients. *Clin. Endocrinol.* 35: 361-367, 1991b.

Ross, R. J. M., and D. W. Wilmore. Endocrinology in catabolic illness. *Endocrinol. Metab.* 3: 115-118, 1996.

Roth, E., J. Funcovics, F. Mühlbacker, M. Schemper, W. Mauritz, P. Sporn, and A. Fritsch. Metabolic disorders in severe abdominal sepsis: glutamine deficiency in skeletal muscle. *Clin. Nutr.* 1: 25-42, 1982.

Rothwell, P. M., and P. G. Lawler. Prediction of outcome in intensive care patients using endocrine parameters. *Crit. Care Med.* 23: 78-83, 1995.

Ruderman, N. B., and M. Berger. The formation of glutamine and alanine in skeletal muscle. *J. Biol. Chem.* 249: 5500-5506, 1974.

Russell-Jones, D. L., A. J. Weissberger, S. B. Bowes, J. M. Kelly, M. Thomason, A. M. Umpleby, R. H. Jones, and P. H. Sönksen. The effects of growth hormone on protein metabolism in adult growth hormone deficient patients. *Clin. Endocrinol.* 38: 427-431, 1993.

Russell-Jones, D. L., A. M. Umpleby, T. R. Hennessy, S. B. Bowes, F. Shojaee-Moradie, K. D. Hopkins, N. C. Jackson, J. M. Kelly, R. H. Jones, and P. H. Sönksen. Use of a leucine clamp to demonstrate that IGF-I actively stimulates protein synthesis in normal humans. *Am. J. Physiol.* 267 (*Endocrinol. Metab.* 30): E591-E598, 1994.

Salomon, F., R. C. Cuneo, R. Hesp, and P.H. Sönksen. The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. *N.E.J.M.* 321: 1797-803, 1989.

Schwenk, W. F., P. J. Berg, B. Beaufrere, J. M. Miles, and M. W. Haymond. Use of t-butyldimethylsilylation in the gas chromatographic/mass spectrometric analysis of physiologic compounds found in plasma using electron-impact ionization. *Anal. Biochem.* 141: 101-109, 1984.

Sergi, G., P. Perini, M. Bussolotto, F. Zurlo, L. Malvasi, R. Carraro, S. Del Prato, V. Giantin , and G. Enzi. Body composition study in the elderly: comparison between tritium dilution method and dual photon absorptiometry. *J. Gerontol.* 48: 244-248, 1993.

Sherwin, R. S., G. A. Schulman, R. Hendler, M. Walesky, A. Belous, and W. Tamborlane. Effect of GH on oral glucose tolerance and circulating metabolic fuels in man. *Diabetologia* 24: 155-161, 1983.

Smith, K. and M. J. Rennie. The measurement of tissue protein turnover. *Baillière's Clinical Endocrinology and Metabolism* 10: 469-495, 1996.

Smith, R. J. and D. W. Wilmore. Glutamine nutrition and requirements. *J.P.E.N.* 14: 94S-99S, 1990.

Soeters, P. B. Glutamine: the link between depletion and diminished gut function ? *J. Am. Coll. Nutr.* 18: 549-556, 1994.

Soeters, P. B, and S. W. M. Olde Damink. Nutritional considerations in the critically ill. *Cur. Op. Crit. Care* 2: 153-160, 1996.

Souba, W.W., R. J. Smith, and D. W. Wilmore. Effects of glucocorticods on glutamine metabolism in visceral organs. *Metabolism* 34: 450-456, 1985.

Souba, W. W. *Glutamine physiology, biochemistry and nutrition in critical illness.* CRC Press, 1992.

Souba, W. W. Nutritional support. *N.E.J.M.* 336: 41-48, 1997.

Sönksen, P. H. Double antibody technique for the simultaneous assay of insulin and growth hormone. *Hormones in Human Blood: Detection and Assay.* ed H. N. Antoniades, Harvard University Press, p176-199, 1976.

Stehle, P., J. Zander, N. Mertes, S. Albers, C. H. Puchstein, P. Lawn, and P. Fürst. Effect of parenteral glutamine peptide supplements on muscle glutamine loss and nitrogen balance after major surgery. *Lancet* 1: 221-233, 1989.

Streat, S. J., A. H. Beddoe, and G. L. Hill. Measurement of body fat and hydration of the fat-free body in health and disease. *Metabolism* 34: 509-518, 1985.

Streat, S. J., A. H. Beddoe, and G. L. Hill. Aggressive nutritional support does not prevent protien loss despite fat gain in septic patients. *J. Trauma* 27: 262-266, 1987.

Szondy, Z., and E. A. Newsholme. The effect of glutamine concentration on the activity of carbamoyl-phosphate synthase II and on incorporation of [^{3}H]thymidine into DNA in rat mesenteric lymphocytes stimulated by phytohaemagglutinin. *Biochem. J.* 261: 979-983, 1989.

Tadros, L. B., N. M. Willhoft, P. M.Taylor, and M. J. Rennie. Effects of glutamine deprivation on glutamine transport and synthesis in primary tissue culture of rat skeletal muscle. *Am. J. Physiol.* (*Endocrinol. Metab.* 28) 265:935-942, 1993.

Takala, J., E. Ruokonen, N. R. Webster, M. S. Nielsen, D. F. Zandstra, G. Vundelinckx, and C. J. Hinds. Increased mortality associated with growth hormone treatment in critically ill adults. *N.E.J.M.* 341: 785-792, 1999.

Timmins, A. C., A. M. Cotterill, S. C. Cwyfan Hughes, J. M. P. Holly, R. J. M. Ross, W. Blum , and C. J. Hinds. Critical illness is associated with low circulating concentrations of insulin-like growth factors-I and -II, alterations in insulin-like growth factor binding proteins and induction of an insulin-like growth factor binding protein 3 protease. *Crit. Care Med.* 24: 1460-1466, 1996.

Tremel, H., B. Kienle, L. S. Weilemann, P. Stehle, and P. Fürst. Glutamine dipeptide-supplemented parenteral nutrition maintains intestinal function in the critically ill. *Gastroenterology* 107: 1595-1601, 1994.

Turkalj, I., U. Keller, R, Ninnis, S. Vosmeer, and W. Stauffacher. Effect of increasing doses of recombinant human IGF-I on glucose lipid and leucine metabolism in man. *J. Clin. Endocrinol. Metab.* 75: 1186-1191, 1992.

Umpleby, A. M., and P. H. Sönksen. Measurement of the turnover of substrates of carbohydrate and protein metabolism using radioactive isotopes. *Baillière's Clinical Endocrinology and Metabolism* 1: 773-796, 1987

Unneberg, K., M. Mjaaland, L. Balteskard, T. G. Jenssen, and A. Revhaug. Growth hormone pre-treatment combined with exogenous glutamine induced a postoperative shift from glucose to glutamine consumption in the gastrointestinal tract. *Clin. Nutr.* 15: 165-170, 1996.

Van Acker, B. A. C., R. R. W. J. Van der Hulst, K. W. E. Hulsewé, M. F. Von Meyenfeldt, N. E. P. Deutz, A. J. M. Wagenmakers, and P. B. Soeters. Glutamine metabolism in humans. *Amino acid and protein metabolism in health and disease.* eds. P. Tessari, P.B. Soeters, G. Pittoni and A. Tienzo, Smith-Gordon, p59-67, 1997.

Van Acker, B. A. C., K. W. E. Hulsewé, A. J. M. Wagenmakers, N. E. P. Deutz, B. K. Van Kreel, D. Halliday, D. E. Matthews, P. B. Soeters, and M. F. Von Meyenfeldt. Absence of glutamine isotopic steady state: implications for the assessment of whole-body glutamine production rate. *Clin. Sci.* 95: 339-346, 1998.

Van Acker, B. A. C., M. F. Von Meyenfeldt, R. R. W. J Van der Hulst, K. W. E. Hulsewé, A. J. M. Wagenmakers, N. E. P. Deutz, I. de Blaauw, C. H. C., Dejong, B. K. Van Kreel, and P. B. Soeters. Glutamine: the pivot of our nitrogen economy ? *J.P.E.N.* 23: 45S-48S, 1999.

Van den Berghe, G., F. de Zegher, and P. Lauwers. Dopamine and the sick euthyroid syndrome in critical illness. *Clin. Endocrinol.* 41: 731-737, 1994a.

Van den Berghe, G., F. de Zegher, P. Lauwers, and J. D. Veldhuis. Growth hormone secretion in critical illness: effect of dopamine. *J. Clin. Endocrinol. Metab.* 79: 1141-1146, 1994b.

Van den Berghe, G., F. de Zegher, and R. Bouillon. Acute and prolonged critical illness as different neuroendocrine paradigms. *J. Clin. Endocrinol. Metab.* 83: 1827-1834, 1998.

Van den Berghe, G. Whither growth hormone in the catabolic state of critical illness ? *GH & IGF Res.* 9:397-399 1999.

Van der Hulst, R. R. W. J., B. K. van Kreel, M. F. Von Meyenfeldt, R.-J. M. Brummer, J.-W. Arends, N. E. P. Deutz, and P. B. Soeters. Glutamine and the preservation of gut integrity. *Lancet* 341: 1363-1365, 1993.

Van der Hulst, R. R. W. J., M. F. Von Meyenfeldt, N. E. P. Deutz, and P. B. Soeters. Glutamine extraction by the gut is reduced in patients with depleted gastrointestinal cancer. *Ann. Surg.* 225: 112-121, 1997.

Varoqui, H., H. Zhu, D. Yao, and J. D. Erickson. Cloning and functional identification of a neuronal glutamine transporter. *J. Biol. Chem.* 275: 4049-4054, 2000.

Vermes, I., A. Bieshuizen, R. M. Hampsink, and C. Haanen. Dissociation of plasma adrenocorticotropin and cortisol levels in critically ill patients: possible role of endothelin and atrial natriuretic hormone. *J. Clin. Endocrinol. Metab.* 80: 1238-1242, 1995.

Vinnars, E., J. Bergstöm, and P. Fürst. Influence of the postoperative state on the intracellular free amino acids in human muscle tissue. *Ann. Surg.* 182: 665-671, 1975.

Walser, M. Misinterpretation of nitrogen balances when glutamine stores fall or are replenished. *Am. J. Clin. Nutr.* 53: 1337-1338, 1991.

Wernerman, J., R. Brandt, T. Strandell, L. G. Allgen, and E. Vinnars. The effect of stress hormones on the interorgan flux of amino acids and on the concentration of free amino acids in skeletal muscle. *Clin. Nutr.* 4: 207-216, 1985.

Wernerman, J., F. Hammarqvist, M. R. Ali, and E. Vinnars. Glutamine and ornithine-α-ketoglutarate but not branched-chain amino acids reduce the loss of muscle glutamine after surgical trauma. *Metabolism* 38: 63S-66S, 1989.

Wernerman, J., F. Hammarqvist, and E. Vinnars. α-ketoglutarate and postoperative muscle catabolism. *Lancet* 335:701-703, 1990.

Wernerman, J., and F. Hammarqvist. Glutamine a necessary nutrient for the intensive care patient. *Int. J. Colorectal Dis.* 14: 137-142, 1999.

Windmueller, H. G., and A. E. Spaeth. Uptake and metabolism of plasma glutamine by the small intestine. *J. Biol. Chem.* 249: 5070-5079, 1974.

Windmueller, H. G., and A. E. Spaeth. Intestinal metabolism of glutamine and glutamate from the lumen as compared to glutamine from the blood. *Arch. Biochem. Biophys.* 171: 662-672, 1975.

Windmueller, H. G., and A. E. Spaeth. Identification of ketone bodies and glutamine as the major respiratory fuels in vivo for postabsorptive rat small intestine. *J. Biol. Chem.* 253: 69-76, 1978.

Windmueller, H. G., and A. E. Spaeth. Respiratory fuels and nitrogen metabolism in vivo in small intestine of fed rats: quantitative importance of glutamine, glutamate and aspartate. *J. Biol. Chem.* 255: 107-122, 1980.

Wolfe, R. R. *Radioactive and Stable Isotope Tracers in Biomedicine: Principles and Practice of Kinetic Analysis.* Wiley-Liss, 1992.

Yang, R. D., D. E. Matthews, D. M. Bier, C. Lo, and V. R. Young. Alanine kinetics in humans: influence of different isotopic tracers. *Am. J. Physiol.* 247 (*Endocrinol. Metab.* 10): E634-E638, 1984.

Young, S. C. J., A. Celniker, L. E. Underwood, and D. R. Clemmons. Effects of recombinant IGF-I and GH treatment upon serum IGF-I binding proteins in calorically restricted adults. *J. Clin. Endocrinol. Metab.* 75: 603-608, 1992.

Young, V. R., and A. Ajami. Isotopes in nutrition research. *Proc. Nutr. Soc.* 58: 15-32, 1999.

Yudkoff, M., I. Nissim, and S. Segal. Determination of [amide-^{15}N]glutamine in plasma with gas chromatography-mass spectrometry. *Clin. Chim. Acta.* 118: 159-166, 1982.

Zeigler, T. R., L. S. Young, J. M. Manson, and D. W. Wilmore. Metabolic effects of recombinant human growth hormone in patients recieving parenteral nutrition. *Ann. Surg.* 208: 6-16, 1988.

Zeigler, T. R., K. Benfell, R. J. Smith, L. S. Young, E. Brown, E. Ferrari-Baliviera, D. K. Lowe, and D. W. Wilmore. Safety and metabolic effects of L-glutamine administration in humans. *J.P.E.N.* 14: 137S-145S, 1990a.

Zeigler, T. R., L. S. Young, E. Ferrari-Baliviera, R. H. Demling, and D. W. Wilmore. Use of human growth hormone combined with nutritional support in a critical care unit. *J.P.E.N.* 14: 574-581, 1990b.

Zeigler, T. R., L. S. Young, K. Benfell, M. Scheltinga, K. Hortos, R. Bye, F. D. Morrow, D. O. Jacobs, R. J. Smith, J. H. Antin, and D. W. Wilmore. Clinical and metabolic efficacy of glutamine-supplemented parenteral nutrition after bone marrow transplantation. *Ann. Intern. Med.* 116: 821-828, 1992.

Zeigler, T. R., R. L. Bye, R. L. Persinger, L. S. Young, J. H. Antin, and D. W. Wilmore. Effects of glutamine supplementation on circulating lymphocytes after bone marrow transplantation: a pilot study. *Am. J. Med. Sci.* 315: 4-10, 1998.

The metabolic consequences of critical illness: acute effects on glutamine and protein metabolism

N. C. JACKSON,[1] P. V. CARROLL,[1] D. L. RUSSELL-JONES,[1]
P. H. SÖNKSEN,[1] D. F. TREACHER,[2] AND A. M. UMPLEBY[1]
Departments of [1]Diabetes, Endocrinology and Metabolic Medicine and [2]Intensive Care, St. Thomas' Hospital, London SE1 7EH, United Kingdom

Jackson, N. C., P. V. Carroll, D. L. Russell-Jones, P. H. Sönksen, D. F. Treacher, and A. M. Umpleby. The metabolic consequences of critical illness: acute effects on glutamine and protein metabolism. *Am. J. Physiol.* 276 (*Endocrinol. Metab.* 39): E163–E170, 1999.—Net protein loss and large decreases in plasma glutamine concentration are characteristics of critical illness. We have used [2-^{15}N]glutamine and [1-^{13}C]leucine to investigate whole body glutamine and leucine kinetics in a group of critically ill patients and matched healthy controls. Glutamine appearance rate ($R_{a,Gln}$) was similar in both groups. However, in the patients, the proportion of $R_{a,Gln}$ arising from protein breakdown was higher than in the control group (43 ± 3 vs. 32 ± 2%, $P <$ 0.05). Glutamine metabolic clearance rate (MCR) was 92 ± 8% higher ($P < 0.001$), whereas plasma glutamine concentration was 38 ± 5% lower ($P < 0.001$) than in the control group. Leucine appearance rate (whole body proteolysis) and nonoxidative leucine disposal (whole body protein synthesis) were 59 ± 14 and 49 ± 15% higher in the patients ($P < 0.001$). Leucine oxidation and MCR were increased in the patients by 104 ± 37 and 129 ± 39%, respectively ($P < 0.05$). These results demonstrate that critical illness is associated with a major increase in protein turnover. The acute decrease in plasma glutamine concentration and the unaltered plasma $R_{a,Gln}$ suggest that the increase in proteolysis is insufficient to meet increased demand for glutamine in this severe catabolic state.

stable isotopes; leucine; age

CRITICAL ILLNESS resulting from trauma, surgery, or sepsis is associated with altered metabolism characterized by an increased catabolic rate, negative nitrogen balance, wasting of lean body mass, immunosuppression, and compromised wound healing. The muscle loss is thought to be due to the mobilization of amino acids for high priority use by organs in the splanchnic area for gluconeogenesis, oxidation, ureagenesis, and protein synthesis and also as substrates for the immune system and wound healing (18).

Glutamine is the most abundant amino acid in both plasma and the free intracellular amino acid pool in skeletal muscle (4). Because most tissues have the ability to synthesize glutamine, it is defined as a nonessential amino acid. However, free glutamine concentrations are extremely labile, and marked decreases have been reported in a variety of catabolic states (3, 37). This suggests that during serious illness a deficiency in glutamine availability may develop and has led to the idea that glutamine is a conditionally essential amino acid (24). At present, glutamine is not routinely added to parenteral nutrition solutions, but recent clinical trials suggest that glutamine supplementation improves both nitrogen balance and gut mucosal integrity and decreases the number of infections and length of hospital stay (36, 42).

The use of [1-^{13}C]leucine as a tracer to measure rates of whole body protein breakdown and synthesis is a well-established technique that has been applied to a variety of clinical conditions (19). The measurement of whole body plasma glutamine flux by use of glutamine labeled with ^{15}N has also been developed, allowing experimental and clinical investigations of glutamine metabolism. Healthy subjects and the effects of various catabolic hormones on glutamine metabolism have been studied (7, 10, 11, 20, 28). However, despite the current clinical interest in the benefits of glutamine supplementation, there have been very few tracer studies investigating glutamine metabolism in patient groups, particularly critically ill patients. To our knowledge there have been no published reports of studies using stable isotope tracer techniques to measure glutamine metabolism in critically ill patients soon after the onset of illness in a general intensive care unit (ICU).

The aim of the present study was to use [2-^{15}N]glutamine and [1-^{13}C]leucine to investigate whole body glutamine and leucine metabolism in a group of critically ill patients and a group of matched healthy controls. The importance of matching the critically ill group for age and weight was also studied by comparing glutamine and leucine kinetics in a young and an elderly control group.

METHODS

Materials

L-[2-^{15}N]glutamine (99% AP), L-[1-^{13}C]leucine (99% AP), and $NaH^{13}CO_3$ (99% AP) were purchased from Tracer Technologies (Somerville, MA). Sterile solutions of the tracers were prepared in 0.9% saline with an aseptic technique by the Pharmacy Department, Guy's and St. Thomas' Hospital (London, UK).

Subjects

Seven severely ill patients (age range 32–76 yr) in the ICU of St. Thomas' Hospital were studied. Details of their clinical and metabolic characteristics are given in Table 1. The severity of illness was evaluated on the day of the study by use of the APACHE II and TISS score systems (Table 1). Five of the patients had undergone emergency abdominal surgery within 24 h before the study. *Patients 3* and *5* had had previous infections, but none of the patients had evidence of

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Table 1. *Clinical and metabolic characteristics of patients*

Patient No.	Gender	Age, yr	Weight, kg	BMI, kg/m²	Main Diagnosis	APACHE II	TISS	Duration Postsurgery,* h	Plasma Albumin, g/dl	Plasma CRP, mg/dl
1	F	68	65	22.2	GI obstruction/laparotomy	15	34	24	19	157
2	M	76	74	22.2	GI bleed/laparotomy, ARF	23	49	24	27	68
3	M	72	82	23.4	Pneumonia, GI obstruction	15	37	NA	23	161
4	F	65	75	26.0	GI infarction/laparotomy	9	50	16	34	82
5	F	75	85	30.1	ARDS/sepsis	19	38	NA	25	329
6	M	75	104	31.0	AAA repair	24	47	20	19	248
7	F	32	59	20.2	Tumor/colonic resection	18	62	16	17	145
Mean ± SE	3M/4F	66 ± 6	78 ± 6	25.0 ± 1.6		18 ± 2	45 ± 4	20 ± 2*	23 ± 2	170 ± 35
Characteristics of matched controls										
Mean ± SE	3M/4F	62 ± 6	78 ± 4	27.1 ± 1.7						

BMI, body mass index; CRP, C-reactive protein; GI, gastrointestinal; ARF, acute renal failure; ARDS, acute respiratory distress syndrome; AAA, aortic abdominal aneurysm; NA, not applicable. *Includes data from 5 patients who had undergone emergency surgery.

active infection (negative blood, sputum, and urine culture) during the study period. All patients were studied after a fasting period of between 12 and 24 h. Six of the seven patients were admitted acutely and had previously been self caring and independent, responsible for their own diet. The remaining patient (*patient 5*) had been receiving an oral hospital diet for 19 days before the study.

A group of 12 control subjects were recruited for the study; their physical characteristics are shown in Table 2. All were in good general health. There was no recent relevant medical history, and none of the controls was on any regular medication. The healthy adults were divided into two groups on the basis of age, young (<35 yr) and elderly (>60 yr).

The protocol was approved by the Ethics Committee, Guy's and St. Thomas' National Health Service Trust. All control subjects provided informed written consent; written consent was obtained from relatives or friends of ICU patients.

Study Protocol

ICU patients. All ICU patients were fasted for ≥12 h before the start of the study. Indwelling arterial and central venous lines were used for blood sampling and for the tracer infusion, respectively. After baseline sampling, priming boluses of [1-^{13}C]leucine (1 mg/kg) and $NaH^{13}CO_3$ (0.2 mg/kg) were injected, and 4-h constant infusions of [2-^{15}N]glutamine (2.5 $mg \cdot kg^{-1} \cdot h^{-1}$) and [1-^{13}C]leucine (1 $mg \cdot kg^{-1} \cdot h^{-1}$) were started. Blood and breath samples were taken at 210, 215, 220, 225, 230, and 240 min for steady-state measurement of plasma glutamine and α-ketoisocaproic acid enrichment, glutamine and leucine concentrations, and breath $^{13}CO_2$ enrichment. Blood samples were also taken at baseline and steady state for the measurement of metabolite and hormone levels [plasma albumin, C-reactive protein, glucose, insulin, insulin-like growth factor I (IGF-I), cortisol, thyroid hormones, and amino acids]. For glutamine analysis, 0.5-ml lithium heparin plasma aliquots were mixed with 100 μl of internal standard (100 nmol/l [U-$^{13}C_5$]glutamine). All samples were stored at −70°C until analysis.

The ICU patients were all sedated and mechanically ventilated (Servo 900; Siemens, Berlin, Germany). Continuous measurements of expired volume, CO_2 production, and oxygen consumption were made using an on-line mass spec-

Table 2. *Characteristics of the healthy volunteers*

Subject No.	Gender	Age, yr	Weight, kg	Height, cm	BMI, kg/m²	LBM, kg	Fat Mass, kg
				Young (<35 yr)			
1	F	31	65.4	167	23.5	47.2	18.2
2	M	27	80.7	184	23.8	68.5	11.9
3	M	23	76.7	183	22.9	65.8	10.9
4	F	25	66	166	24.0	45.7	20.3
5	F	29	62	171	21.2	44.8	17.2
6	M	34	68	170	23.5	44.1	23.9
Mean ± SE		28 ± 2	69.8 ± 3.0	173.5 ± 3.3	23.2 ± 0.4	52.7 ± 4.6	17.1 ± 2.2
				Elderly (>60 yr)			
7	F	65	78.9	157.5	31.8	41.5	37.4
8	M	70	91.5	174	30.2	62.8	28.7
9	F	67	76.2	156	31.3	29.5	46.7
10	M	65	86.6	185	25.3	69.2	17.4
11	M	77	85.5	174	28.2	62.0	23.5
12	F	62	62.8	170	21.7	44.0	18.8
Mean ± SE		68 ± 2	80.3 ± 4.2	169.4 ± 4.5	28.1 ± 1.6	51.5 ± 6.3	28.8 ± 4.7

LBM, measured lean body mass.

trometer (Airspec 2200; Airspec, Kent, UK). Five measurements were averaged at steady state for each patient.

Healthy subjects. After an overnight fast, the healthy subjects were admitted to the research area of the Diabetes and Endocrine Day Centre (St. Thomas' Hospital). Height and weight were recorded and body composition was measured using the technique of bioelectrical impedance (Tanita, Tokyo, Japan) (25). Cannulas were inserted into an antecubital vein for isotope infusion and a superficial vein of the contralateral hand for blood sampling. During the sampling period, the hand was placed in a heated box (air temperature 60°C) to produce arterialized venous blood (1). An infusion protocol identical to that of the ICU patients was used.

Total CO_2 production, resting energy expenditure, and oxygen consumption were measured at steady state with indirect calorimetry (Medgraphics, Cardiokinetics, Salford, UK).

Experimental Methods

The isotopic enrichment and concentration of glutamine were determined from the *tert*-butyldimethylsilyl derivative by use of a method modified from Wolfe (41). Glutamine concentration was determined by reverse isotope dilution with L-[U-$^{13}C_5$]glutamine (Bioquote, North Yorkshire, UK) as the internal standard. Analysis by gas chromatography-mass spectrometry (GC-MS; MSD 5971A, Hewlett-Packard, Berkshire, UK) used electron impact ionization with selected ion monitoring of the $[M\text{-butyl}]^+$ ions at mass-to-charge ratios of m/z 432, 433, and 436. The isotopic enrichment of α-ketoisocaproate (α-KIC) was measured as the quinoxalinol-*tert*-butyldimethylsilyl derivative by use of a method modified from Ford et al. (16). GC-MS analysis used electron impact ionization with selected ion monitoring of the $[M\text{-butyl}]^+$ ions at m/z 259 and 260. Plasma α-KIC enrichment is used as a measure of intracellular leucine enrichment (29). $^{13}CO_2$ enrichment was measured on a SIRA series II isotope ratio mass spectrometer (VG Isotech, Cheshire, UK) modified with a Roboprep G+ inlet system (Europa Scientific, Cheshire, UK). Plasma amino acid concentrations were measured on an Alpha II+ automated amino acid analyzer (Pharmacia, Hertfordshire, UK). Plasma glucose concentrations were measured on a model 23AM glucose analyzer (YSI, Hampshire, UK). Serum insulin concentrations were measured by an in-house double-antibody radioimmunoassay (34). Total IGF-I was measured by radioimmunoassay after acid ethanol extraction. Free thyroid hormones were measured by a competitive immunoassay using chemiluminesence (Chiron Diagnostics, Essex, UK). Cortisol was measured by ELISA using the Enzymun-Test cortisol kit (Boehringer Mannheim, Sussex, UK). Plasma albumin and C-reactive protein were measured using an automated method (Kodak 250, Ortho Clinical Diagnostics, Amersham, UK).

Calculations

Measurements of leucine and glutamine metabolism were calculated using standard isotope dilution equations. Leucine appearance rate, a measure of whole body protein breakdown ($R_{a,Leu}$; in $\mu mol \cdot min^{-1} \cdot kg^{-1}$) was calculated as $R_{a,Leu} = F[1/(APE_{KIC} \times 0.01) - 1]$, where F is the isotope infusion rate (in $\mu mol \cdot min^{-1} \cdot kg^{-1}$) and APE_{KIC} is the plasma α-KIC enrichment. At steady state, leucine disappearance rate ($R_{d,Leu}$) was assumed to be equal to $R_{a,Leu}$. Leucine oxidation rate (Ox_{Leu}; in $\mu mol \cdot min^{-1} \cdot kg^{-1}$) was calculated as $Ox_{Leu} = (APE_{CO_2} \times R_{a,CO_2})/ APE_{KIC}$, where R_{a,CO_2} is the production rate of CO_2 (in mmol/min), and APE_{CO_2} is the enrichment of expired CO_2. In all cases, leucine oxidation was corrected with the assumption that 80% of $^{13}CO_2$ was expired (32). Nonoxidative leucine disposal (NOLD, a measure of whole body protein synthesis) was calculated as the difference between $R_{d,Leu}$ and Ox_{Leu}. Leucine metabolic clearance rate (MCR_{Leu}; in $ml \cdot min^{-1} \cdot kg^{-1}$) was calculated as $MCR_{Leu} = R_{d,Leu}/[Leu]$, where [Leu] is the steady-state plasma leucine concentration (in μmol/l). Net protein balance was estimated using the net leucine balance (NOLD − R_a), with the assumption of 8 g of leucine for 100 g whole body protein (21).

Glutamine R_a, R_d, and MCR values were calculated using analogous equations. Because glutamine is a nonessential amino acid, $R_{a,Gln}$ is derived from both protein breakdown (B_{Gln}) and de novo synthesis (D_{Gln}). Glutamine release from protein breakdown was estimated as $0.78 \times R_{a,Leu}$ (14), and D_{Gln} was calculated as $D_{Gln} = R_{a,Gln} - B_{Gln}$ (21).

Statistics

All data are presented as means ± SE. Steady state for plasma glutamine enrichment and concentration was confirmed as an insignificant correlation with time ($P > 0.05$) by use of repeated-measures ANOVA (NCSS 6.0, Dr. J. Hintze, Kaysville, UT). Comparisons between groups were made by standard two-tailed unpaired *t*-tests with equal or unequal variance as necessary. The cortisol and insulin data were log transformed before analysis.

RESULTS

ICU Patients vs. Matched Controls

Table 1 shows the details of the seven ICU patients studied. The severity of the illness is indicated by the TISS and APACHE II scores (9, 23). These indexes identified the patients as being severely ill and dependent on cardiorespiratory and nutritional support. The seven healthy volunteers selected as controls were well-matched to the ICU patients for sex, age, weight, and body mass index (BMI, Table 1). Figure 1 shows the plasma glutamine enrichments and concentrations for the ICU patients and their matched controls during the final 30 min of the tracer infusion. The glutamine and leucine data for the ICU patients and their matched controls are summarized in Figs. 2 and 3. Glutamine MCR was significantly higher in ICU patients compared with the matched controls ($P < 0.001$). There was no difference in whole body $R_{a,Gln}$ (or $R_{d,Gln}$) between the two groups. However, there was a significant increase ($P < 0.05$) in the proportion of $R_{a,Gln}$ arising from protein breakdown and a resulting decrease in the proportion arising from de novo synthesis in the critically ill patients ($P < 0.05$).

$R_{a,Leu}$, Ox_{Leu}, MCR_{Leu}, and NOLD were all significantly higher in the critically ill patients ($P < 0.001$, $P < 0.05$, $P < 0.05$, and $P < 0.001$, respectively). Net 24-h protein balance (Fig. 4) was significantly more negative in the critically ill subjects ($P < 0.01$).

The plasma amino acid profiles are given in Table 3. Plasma threonine ($P < 0.01$), serine ($P < 0.001$), glutamine ($P < 0.001$), glycine ($P < 0.01$), alanine ($P < 0.001$), leucine ($P < 0.05$), lysine, ($P < 0.01$), histidine ($P < 0.01$), and arginine ($P < 0.01$) concentrations were significantly lower in the patients, whereas phenylalanine ($P < 0.01$) and aspartate ($P < 0.01$) levels were significantly higher. Metabolite and hormone profiles

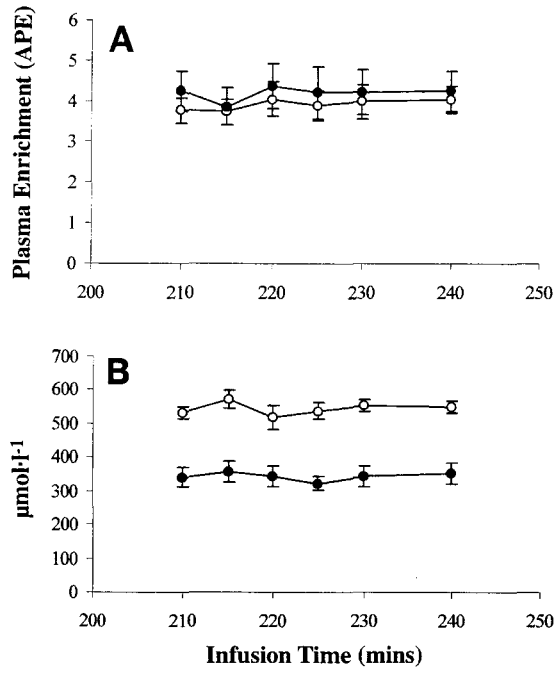


Fig. 1. Plasma glutamine enrichment (APE; *A*) and concentration (μmol/l; *B*) for intensive care unit (ICU) patients (●) and matched controls (○). Values are means ± SE; $n = 7$ in each group.

are shown in Table 4. Thyroid hormone and IGF-I levels were significantly lower ($P < 0.05$) in the patients. There was no difference in the plasma glucose or insulin levels between the two groups. Cortisol levels were higher in the ICU patients ($P = 0.069$, range 242–2,393 nmol/l).

Control Subjects

The characteristics of the control subjects are shown in Table 2. They were divided into two groups on the basis of age; both groups contained three males and three females. The elderly (>60) group had a mean age of 68 ± 2 yr, whereas the young (<35) group had a mean age of 28 ± 2 yr. The mean weights of the groups were 80.3 ± 4.2 and 69.8 ± 3.0 kg, respectively ($P = 0.068$). BMI and fat mass were significantly higher in the elderly group, 28.1 ± 1.60 vs. 23.2 ± 0.42 kg/m^2 ($P < 0.05$) and 28.8 ± 4.7 vs. 17.1 ± 2.2 kg ($P < 0.05$), respectively. Lean body mass (LBM) was not significantly different between the two groups (51.5 ± 6.3 vs. 52.7 ± 4.6 kg).

Table 5 summarizes the glutamine and leucine data from the healthy volunteers. Whole body plasma glutamine flux ($R_{a,Gln}$) was significantly lower in the elderly group (4.15 ± 0.33 vs. 5.20 ± 0.22 μmol·min^{-1}·kg^{-1}, $P < 0.05$), but there was no difference in the proportion of $R_{a,Gln}$ arising from de novo glutamine synthesis or protein breakdown in the two groups. However, when the results were expressed per kilogram LBM, the difference in $R_{a,Gln}$ was no longer evident (6.96 ± 0.37 vs. 6.69 ± 0.51 μmol·min^{-1}·kg LBM^{-1}). There were no significant differences in glutamine MCR or in any of the measurements of leucine metabolism ($R_{a,Leu}$, Ox_{Leu}, MCR_{Leu}, and NOLD) in the two groups of volunteers, whether the results were expressed per kilogram body weight or per kg LBM.

Table 3 shows that plasma amino acid profiles were similar in both groups of healthy volunteers, with the exception of decreased circulating serine ($P < 0.05$) and histidine ($P < 0.05$) and increased plasma cystine concentrations in the elderly subjects. Metabolite and hormone profiles are shown in Table 4. Plasma glucose levels were significantly higher ($P < 0.05$) in the elderly volunteers. Cortisol levels were significantly higher ($P < 0.05$), and IGF-I levels were significantly lower ($P < 0.05$) in the elderly group, but there were no differences in insulin or thyroid hormone levels.

DISCUSSION

There is currently intense clinical interest in glutamine metabolism in critical illness. Previous studies have reported whole body glutamine turnover measurements in healthy controls, burn patients (20), enterectomized patients (12), and patients with insulin-dependent diabetes (13), but there have been no studies investigating glutamine metabolism in acute critical illness. In this study we have shown that, despite a marked decrease in plasma glutamine concentration, whole body plasma glutamine flux was unchanged in critically ill patients. Measured whole body gluta-

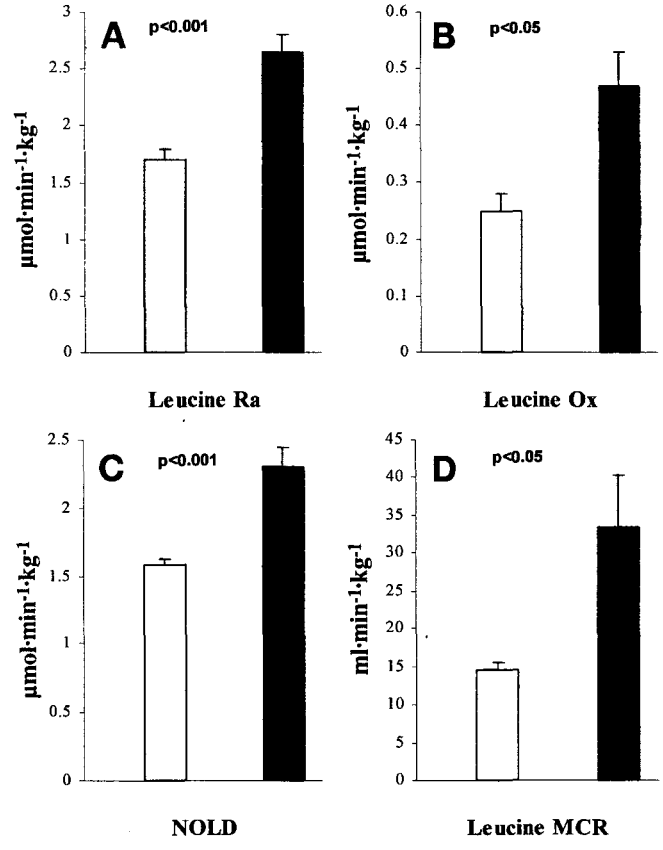


Fig. 2. Leucine appearance rate (R_a, *A*), oxidation rate (Ox, *B*), nonoxidative leucine disposal (NOLD, *C*), and metabolic clearance rate (MCR, *D*) for ICU patients (solid bars) and matched controls (open bars). Values are means ± SE; $n = 7$ in each group.

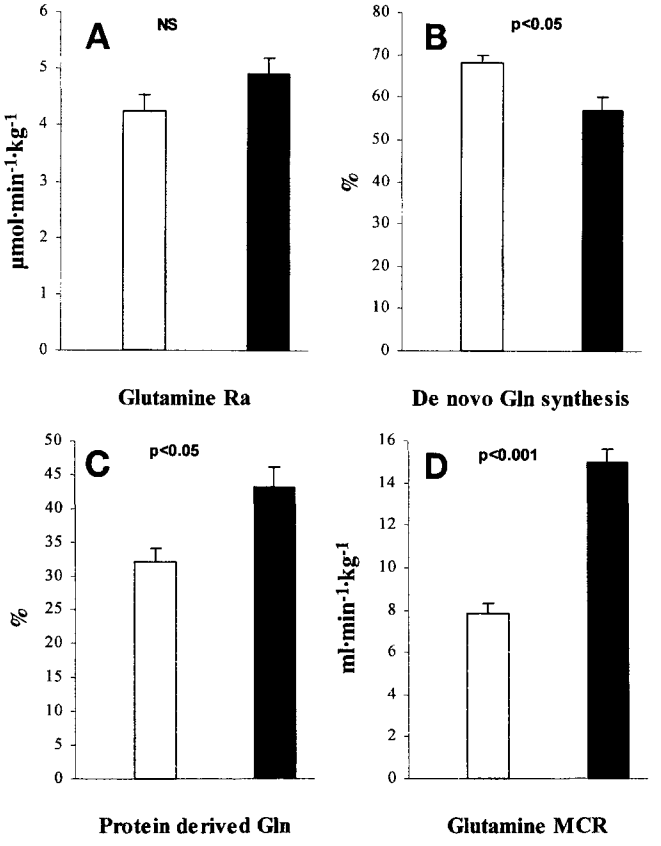


Fig. 3. Glutamine appearance rate (R_a, *A*), %glutamine flux arising from de novo synthesis (*B*), %glutamine flux arising from proteolysis (*C*), and MCR (*D*) for ICU patients (solid bars) and matched controls (open bars). Values are means ± SE; n = 7 in each group. NS, not significant.

mine flux reflects interorgan glutamine transport rates through plasma (10).

Glutamine MCR was increased in the patients, suggesting that this may be the primary mechanism for the fall in glutamine concentration. Because amino acids are removed from blood by a transporter, we would expect this removal to exhibit Michaelis-Menten kinetics (i.e., nonlinear kinetics). There will thus be an inverse relationship between clearance and concentration. Thus, if R_a decreases, concentration will fall and clearance will rise. However, glutamine R_a-to-R_d ratio was unchanged in the ICU patients despite a fall in glutamine concentration. The increase in glutamine MCR in the ICU patients must therefore be due to a change in the transport process, e.g., an increase in efficiency.

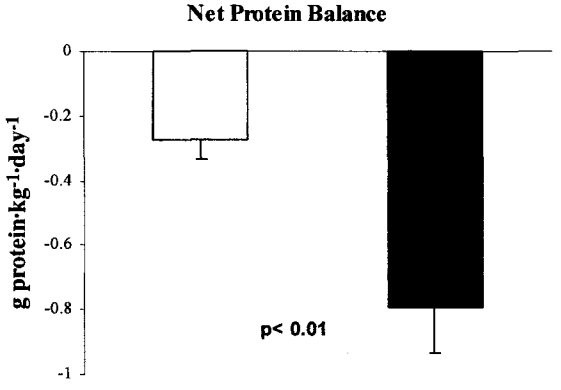


Fig. 4. Net 24-h protein balance in ICU patients (solid bar) and matched controls (open bar). Values are means ± SE; n = 7 in each group.

The percentage of the glutamine flux arising from protein breakdown was increased in this patient group, but the observed decrease in plasma glutamine concentration indicates that this increase was insufficient to meet the increased demand for glutamine. Marked alterations were also observed in protein metabolism, as reflected by the increases in leucine R_a, NOLD, MCR, and oxidation. These changes resulted in an increased net negative protein balance in the critically ill patients.

Marked differences were observed in the circulating levels of amino acids between the patients and their matched controls. In addition to the decreased plasma glutamine concentration, the levels of the essential amino acids leucine, lysine, threonine, and histidine were lower in the patients. Similar changes have been observed in previous studies (18, 33), and it has been suggested that elevated levels of stress hormones, by increasing splanchnic amino acid uptake, may be responsible for the decreased plasma amino acid concentrations (39). The decrease in glutamine levels may indicate an inability of glutamine synthetic mechanisms to meet the increased metabolic demand of critical illness. These findings have led to the suggestion that glutamine may behave as a "conditionally essential" amino acid. In contrast, there was an increase in the plasma phenylalanine concentration. This response has been observed in previous studies, and evidence suggests that levels of phenylalanine continue to rise with continuing illness (18, 33).

The metabolic response to critical illness is an integrated process, with elevated levels of cytokines and inflammatory mediators and increased concentrations of the "catabolic" hormones (catecholamines, glucocorticoids, and glucagon) (35). Changes observed in the patients in this study included elevated plasma cortisol concentration and decreased levels of free thyroid hormones.

Previous studies have investigated the effects of catabolic hormone infusions on glutamine metabolism in healthy volunteers. These have demonstrated that elevation of plasma cortisol to levels observed after trauma resulted in a 15% increase in whole body protein breakdown and a 40% increase in glutamine flux (11). This increase in flux was primarily due to a 55% increase in de novo glutamine synthesis, and it resulted in a significant increase in the plasma glutamine concentration. More recently, this cortisol-mediated increase in flux was shown to be dose dependent (7). In contrast, a triple hormone infusion of epinephrine, cortisol, and glucagon increased whole body glutamine flux and MCR and decreased plasma glutamine concentration (20). These studies suggest that these counterregulatory hormones may regulate the rate of glutamine metabolism, possibly through effects on glutamine transporters (30); however, they cannot fully

Table 3. *Plasma amino acid profiles*

Amino Acid, mmol/l	Volunteers		P	Matched Controls	Critically Ill Patients	P
	Young	Elderly				
Aspartate	6 ± 1	6 ± 1	NS	5 ± 0.5	17 ± 6	<0.01
Threonine	121 ± 7	120 ± 21	NS	122 ± 18	48 ± 6	<0.01
Serine	115 ± 6	91 ± 5	<0.01	98 ± 8	50 ± 6	<0.001
Glutamate	44 ± 6	61 ± 10	NS	59 ± 9	34 ± 8	NS
Glutamine	581 ± 35	534 ± 23	NS	540 ± 53	329 ± 21	<0.001
Glycine	204 ± 9	202 ± 22	NS	204 ± 18	129 ± 13	<0.01
Alanine	216 ± 20	283 ± 25	NS	276 ± 23	139 ± 15	<0.001
Valine	198 ± 17	215 ± 18	NS	205 ± 18	151 ± 20	NS
Cystine	50 ± 1	68 ± 4	<0.01	65 ± 5	56 ± 13	NS
Methionine	18 ± 1	33 ± 11	NS	30 ± 9	12 ± 2	NS
Isoleucine	42 ± 4	36 ± 6	NS	34 ± 5	33 ± 13	NS
Leucine	125 ± 12	134 ± 9	NS	129 ± 9	95 ± 12	<0.05
Tyrosine	38 ± 4	39 ± 9	NS	39 ± 7	40 ± 6	NS
Phenylalanine	41 ± 3	44 ± 5	NS	42 ± 4	72 ± 7	<0.01
Ornithine	42 ± 4	46 ± 4	NS	44 ± 4	34 ± 3	NS
Lysine	144 ± 10	166 ± 17	NS	159 ± 16	88 ± 6	<0.01
Histidine	83 ± 8	63 ± 4	<0.05	63 ± 3	39 ± 4	<0.01
Arginine	74 ± 6	64 ± 6	NS	63 ± 5	30 ± 5	<0.01

NS, not significant.

mimic the complex changes occurring in critically ill patients.

Although there are no comparable studies in ICU patients, whole body glutamine flux has been measured in patients after burn injury by use of a similar stable isotope technique (20). Whole body glutamine flux was higher in burns patients compared with the values we obtained (7.2 ± 0.6 vs. 4.9 ± 0.3 $\mu mol \cdot min^{-1} \cdot kg^{-1}$). Although the decreases in plasma glutamine concentration were similar in the two studies, a significant increase (60%) in glutamine flux was reported in the burns patients compared with a control group, in contrast to the unchanged value in the present study. These changes were associated with a marked elevation in glutamine MCR in the burns patients (200%) compared with control subjects, in contrast to the 92% increase recorded in our patient group. However, these measurements were made 2 wk after the burn injury, whereas the patients in the present study were studied within days of admission to the ICU.

The leucine kinetic data in the critically ill indicated an increase in whole body protein synthesis and breakdown of 49 and 59%, respectively, and an increase in leucine oxidation (105%), indicating use of protein as an oxidative fuel. In addition, the plasma leucine concentration was decreased as a result of the increased utilization of leucine (indicated by the elevated leucine MCR). Isotope tracer methdology has been used to investigate whole body protein turnover in a variety of catabolic states, with conflicting results. A study in patients with multiple organ failure, using [1-^{13}C]leucine, demonstrated significant increases in protein

Table 4. *Hormone and metabolite concentrations*

	Units of Measure	Volunteers <35 (n = 6)	Volunteers >60 (n = 6)	P
Glucose	mmol/l	5.1 ± 0.1	5.8 ± 0.3	<0.05
Insulin	mU/l	7 ± 1	12 ± 3	NS
IGF-I	nmol/l	22.3 ± 1.1	16.5 ± 1.7	<0.05
Cortisol	nmol/l	205 ± 16	342 ± 40	<0.05
Free Thyroxine	pmol/l	14.8 ± 0.4	14.7 ± 0.6	NS
Free Triiodothyronine	pmol/l	4.0 ± 0.4	4.6 ± 0.2	NS
		Healthy Controls (n = 7)	Critically Ill Patients (n = 7)	P
Glucose	mmol/l	5.7 ± 0.2	6.0 ± 0.5	NS
Insulin	mU/l	11 ± 3	18 ± 3	NS
IGF-I	nmol/l	17.0 ± 1.5	12.4 ± 0.7	<0.05
Cortisol	nmol/l	318 ± 41	784 ± 288	NS
Free Thyroxine	pmol/l	14.5 ± 0.6	11.4 ± 1.2	<0.05
Free Triiodothyronine	pmol/l	4.6 ± 0.2	3.0 ± 0.5	<0.05

IGF-I, insulin-like growth factor I.

Table 5. *Whole body glutamine and leucine kinetics in healthy volunteers*

	Units of Measure	Volunteers <35 (n = 6)	Volunteers >60 (n = 6)	P
Glutamine R_a	$\mu mol \cdot min^{-1} \cdot kg^{-1}$	5.20 ± 0.22	4.15 ± 0.33	<0.05
De novo glutamine synthesis	%	71 ± 2	67 ± 2	NS
Glutamine derived from proteolysis	%	29 ± 2	33 ± 2	NS
Glutamine MCR	$ml \cdot min^{-1} \cdot kg^{-1}$	9.03 ± 0.40	7.77 ± 0.55	NS
Leucine R_a	$\mu mol \cdot min^{-1} \cdot kg^{-1}$	1.89 ± 0.17	1.71 ± 0.11	NS
Leucine Ox	$\mu mol \cdot min^{-1} \cdot kg^{-1}$	0.28 ± 0.03	0.27 ± 0.02	NS
NOLD	$\mu mol \cdot min^{-1} \cdot kg^{-1}$	1.74 ± 0.16	1.58 ± 0.10	NS
Leucine MCR	$ml \cdot min^{-1} \cdot kg^{-1}$	15.36 ± 1.52	13.98 ± 0.94	NS

R_a, appearance rate; MCR, metabolic clearance rate; Ox, oxidation; NOLD, nonoxidative leucine disposal.

breakdown, synthesis, and leucine oxidation compared with control subjects (2). Plasma cortisol concentration was found to be the most significant predictor of protein breakdown and leucine oxidation in these patients.

In contrast, studies of protein metabolism after elective hysterectomy have shown that both whole body protein synthesis and breakdown decrease compared with the preoperative state (8). Ribosomal analysis and tracer studies have also shown that muscle protein synthesis decreases after uncomplicated elective surgery (15, 31, 40). With use of [^{15}N]alanine as a tracer, a 37% increase in protein synthesis and a 79% increase in protein breakdown have been reported in fed patients 3–5 days after multiple skeletal trauma compared with controls receiving a similar diet (5). More recently, it has been shown that albumin and fibrinogen synthesis increased, whereas muscle protein synthesis decreased, in fed head-trauma patients (27). It is likely that the apparently conflicting data from these studies reflect the heterogeneity of the patient populations, the severity of illness, the nutritional status, the prior health of the patients, and the timing of the studies.

The major limitation of the whole body protein measurement is that this approach reflects the average rates of protein turnover in all tissues. However, during times of severe stress, different tissues in the body may behave differently. The majority of the measured increases in proteolysis may reflect increased muscle protein breakdown, as skeletal muscle is the largest protein pool in the human body. It is likely that, in part, the increase in protein synthesis reflects the increased synthesis of acute-phase proteins by the liver, tissue repair, and the immune response (leukocyte proliferation and cytokine production). This alteration of protein balance would account for the clinical observations of lean tissue loss in ICU patients.

The importance of matching the critically ill group for age and weight was studied by comparing glutamine and leucine kinetics in a young and an old control group. Leucine MCR, oxidation rate, incorporation into and release from body protein were similar in the control groups. This is in line with previously reported data suggesting that there is no independent effect of age on the measurements of leucine metabolism in postabsorptive adults, whether the results are expressed per kilogram body weight (17) or per kilogram LBM (6, 17, 38). The $R_{a,Gln}$ value in the young control subjects was similar to previously published values for healthy adults in the same age range (e.g., 7, 11, 29). In contrast, the $R_{a,Gln}$ value for the elderly group of controls was significantly lower than that in the young controls. However, the increase in body weight in the elderly group resulted from an increase in fat mass, not a decrease in LBM. When the results were expressed per kilogram LBM, there was no age-associated decrease in $R_{a,Gln}$, suggesting that the apparent difference in R_a is related to age-associated changes in body composition rather than altered glutamine metabolism.

It is difficult to measure body composition accurately in ICU patients. Bioelectric impedance is the most accessible method, and this was used in the controls in the present study. However, as there is some doubt about the practicality and validity of bioelectrical impedance measurements of body composition in critically ill patients (22, 26), these results suggest that matched controls are necessary when glutamine metabolism is measured in ICU patients.

The patients for our study were recruited in the ICU from patients in whom the clinical decision had been made to use parenteral nutrition. Unlike previous studies reporting glutamine metabolism and most of the studies of leucine metabolism in catabolic patients, we have studied a heterogeneous group. We chose to study these patients because they represent a group in whom there is considerable clinical interest in the potential benefits of glutamine supplementation. The study demonstrates that critical illness is associated with marked alterations in protein metabolism. The increased glutamine clearance with a normal $R_{a,Gln}$ resulted in a decrease in glutamine concentration, suggesting that the increase in protein breakdown was insufficient to meet the demand for glutamine in these catabolic patients.

We are grateful to the nurses and staff of Mead Ward (ICU), St Thomas' Hospital, for their patience and support. We acknowledge the assistance of Dr. F. Shojaee-Moradie, S. Imuere, and M. Chaudhury, and the Department of Chemical Pathology, with sample analysis. We also thank P. Forsey, of the hospital pharamacy, for preparation of the tracer solutions.

Address for reprint requests: N. Jackson, Dept. of Diabetes, Endocrinology and Metabolic Medicine, Fourth Floor North Wing, St. Thomas' Hospital, London SE1 7EH, UK.

Received 30 April 1998; accepted in final form 15 September 1998.

REFERENCES

1. **Abumard, N. N., D. Rabin, M. P. Diamond, and W. W. Lacy.** Use of a heated superficial hand vein as an alternative site for the measurement of amino acid concentrations for the study of glucose and alanine kinetics in man. *Metabolism* 30: 936–940, 1982.
2. **Arnold, J., I. T. Campbell, T. A. Samuels, J. C. Devlin, C. J. Green, L. J. Hipkin, I. A. Macdonald, C. M. Scrimgeour, K. Smith, and M. J. Rennie.** Increased whole body protein breakdown predominates over whole body protein synthesis in multiple organ failure. *Clin. Sci. (Colch.)* 84: 655–666, 1993. [Errors in *Clin. Sci. (Colch.)* 85: following xxv].
3. **Askanazi, J., Y. A. Carpentier, C. B. Michelsen, D. H. Elwyn, P. Furst, L. R. Kantrowitz, F. E. Gump, and M. J. Kinney.** Muscle and plasma amino acids following injury: influence of intercurrent infection. *Ann. Surg.* 192: 78–85, 1980.
4. **Bergström, J., P. Fürst, L.-O. Norée, and E. Vinnars.** Intracellular free amino acid concentration in human muscle tissue. *J. Appl. Physiol.* 36: 693–697, 1974.
5. **Birkhahn, R. H., C. L. Long, D. Fitkin, M. Jeevanandam, and W. S. Blakemore.** Whole-body protein metabolism due to trauma in man as estimated by L-[^{15}N]alanine. *Am. J. Physiol.* 241 (*Endocrinol. Metab.* 4): E64–E71, 1981.
6. **Boirie, Y., P. Gachon, and B. Beaufrere.** Splanchnic and whole body leucine kinetics in young and elderly men. *Am. J. Clin. Nutr.* 65: 489–495, 1996.
7. **Brillon, D. J., B. Zheng, R. G. Campbell, and D. E. Matthews.** Effect of cortisol on energy expenditure and amino acid metabolism in humans. *Am. J. Physiol.* 268 (*Endocrinol. Metab.* 31): E501–E513, 1995.
8. **Carli, F., V. Ramachandra, J. Gandy, H. Merrit, G. C. Ford, M. Read, and D. Halliday.** Effect of general anaesthesia on whole body protein turnover in patients undergoing elective surgery. *Br. J. Anaesth.* 65: 373–379, 1990.

9. **Cullen, D. J., J. M. Civetta, B. A. Briggs, and L. C. Ferrara.** Therapeutic intervention scoring system: a method for quantitative comparison of patient care. *Crit. Care Med.* 2: 57–60, 1974.
10. **Darmaun, D., D. E. Matthews, and D. M. Bier.** Glutamine and glutamate kinetics in humans. *Am. J. Physiol.* 251 (*Endocrinol. Metab.* 14): E117–E126, 1986.
11. **Darmaun, D., D. E. Matthews, and D. M. Bier.** Physiological hypercortisolemia increases proteolysis, glutamine, and alanine production. *Am. J. Physiol.* 255 (*Endocrinol. Metab.* 18): E366–E373, 1988.
12. **Darmaun, D., B. Messing, B. Just, M. Rongier, and J.-F. Desjeux.** Glutamine metabolism after small intestinal resection in humans. *Metabolism* 40: 42–44, 1991.
13. **Darmaun, D., M. Rongier, J. Koziet, and J.-J. Robert.** Glutamine nitrogen kinetics in insulin-dependent diabetic humans. *Am. J. Physiol.* 261 (*Endocrinol. Metab.* 24): E713–E718, 1991.
14. **Darmaun, D., S. Welch, A. Rini, B. K. Sager, A. Altomare, and M. W. Haymond.** Phenylbutyrate-induced glutamine depletion in humans: effect on leucine metabolism. *Am. J. Physiol.* 274 (*Endocrinol. Metab.* 37): E801–E807, 1998.
15. **Essen, P., M. A. McNurlan, J. Wernerman, E. Vinnars, and P. J. Garlick.** Uncomplicated surgery, but not general anesthesia, decreases muscle protein synthesis. *Am. J. Physiol.* 262 (*Endocrinol. Metab.* 25): E253–E260, 1992.
16. **Ford, G. C., K. N. Cheng, and D. Halliday.** The analysis of (1-^{13}C)leucine and (^{13}C)KIC in plasma by capillary gas chromatography mass spectrometry in protein turnover studies. *Biomed. Mass Spectrom.* 12: 432–436, 1985.
17. **Fukagawa, N. K., K. L. Minaker, V. R. Young, D. E. Matthews, D. M. Bier, and J. W. Rowe.** Leucine metabolism in aging humans: effect of insulin and substrate availability. *Am. J. Physiol.* 256 (*Endocrinol. Metab.* 19): E288–E294, 1989.
18. **Gamrin, L., P. Essen, A. M. Forsberg, E. Hultman, and J. Wernerman.** A descriptive study of skeletal muscle metabolism in critically ill patients: free amino acids, energy rich phosphates, protein, nuceic acids, fat, water and electrolytes. *Crit. Care Med.* 24: 575–583, 1996.
19. **Garlick, P. J., and E. Cersosimo.** Techniques for assessing protein and glucose kinetics. *Baillieres Clin. Endocrinol. Metab.* 11: 629–644, 1997.
20. **Gore, D. C., and F. Jahoor.** Glutamine kinetics in burn patients, comparison with hormonally induced volunteers. *Arch. Surg.* 129: 1318–1323, 1994.
21. **Hankard, R. G., D. Darmaun, B. K. Sager, D. D'Amore, W. R. Parsons, and M. Haymond.** Response of glutamine metabolism to exogenous glutamine in humans. *Am. J. Physiol.* 269 (*Endocrinol. Metab.* 32): E663–E670, 1995.
22. **Jacobs, D. O.** Use of bioelectrical impedance analysis measurements in the clinical management of critical illness. *Am. J. Clin. Nutr.* 64: 498S–502S, 1996.
23. **Knaus, W. A., E. A. Draper, D. P. Wagner, and J. E. Zimmerman.** APACHE II: a severity of disease classification system. *Crit. Care Med.* 13: 818–829, 1985.
24. **Lacy, J. M., and D. W. Wilmore.** Is glutamine a conditionally essential amino acid? *Nutr. Rev.* 48: 297–309, 1990.
25. **Lukaski, H. C., W. W. Bolonchuk, C. B. Hall, and W. A. Siders.** Validation of tetrapolar bioelectrical impedance method to assess human body composition. *J. Appl. Physiol.* 60: 1327–1332, 1986.
26. **Manning, E. M. C., and A. Shenkin.** Nutritional assessment in the critically ill. *Crit. Care Clin.* 11: 603–634, 1995.
27. **Mansoor, O., M. Cayol, P. Gachon, Y. Biorie, P. Schoeffler, C. Obled, and B. Beaufrère.** Albumin and fibrinogen syntheses increase while muscle protein synthesis decreases in head injured patients. *Am. J. Physiol.* 273 (*Endocrinol. Metab.* 36): E898–E902, 1997.
28. **Matthews, D. E., G. Pesola, and R. G. Campbell.** Effect of epinephrine on amino acid and energy metabolism in humans. *Am. J. Physiol.* 258 (*Endocrinol. Metab.* 21): E948–E956, 1990.
29. **Matthews, D. E., H. P. Schwartz, R. D. Yang, K. J. Motil, V. R. Young, and D. M. Bier.** Relationship of plasma leucine and α-ketoisocaproate during a L-[1-^{13}C]leucine infusion in man: a method for measuring intracellular leucine tracer enrichment. *Metabolism* 31: 1105–1112, 1982.
30. **McGivan, J. D., and M. Pastor-Anglada.** Regulatory and molecular aspects of mammalian amino acid transport. *Biochem. J.* 299: 321–334, 1994.
31. **Petersson, B., J. Wernerman, S. O. Waller, A. von der Decken, and E. Vinnars.** Elective abdominal surgery depresses muscle protein synthesis and increases subjective fatigue: effects lasting more than 30 days. *Br. J. Surg.* 77: 796–800, 1990.
32. **Rodriguez, N., W. F. Schwenk, B. Beaufrere, J. M. Miles, and M. W. Haymond.** Trioctanoin infusion increases in vivo leucine oxidation: a lesson in isotope modeling. *Am. J. Physiol.* 251 (*Endocrinol. Metab.* 14): E343–E348, 1986.
33. **Roth, E., J. Funcovics, F. Mühlbacker, M. Schemper, W. Mauritz, P. Sporn, and A. Fritsch.** Metabolic disorders in severe abdominal sepsis: glutamine deficiency in skeletal muscle. *Clin. Nutr.* 1: 25–42, 1982.
34. **Sönksen, P. H.** *Hormones in Human Blood: Detection and Assay.* Cambridge, MA: Harvard University Press, 1976, p. 176–199.
35. **Van den Berghe, G., F. De Zegher, and R. Bouillon.** Acute and prolonged critical illness as different neuroendocrine paradigms. *J. Clin. Endocrinol. Metab.* 83: 1827–1834, 1998.
36. **Van der Hulst, R. R., B. K. van Kreel, M. F. von Meyenfeldt, R. J. M. Brummer, J.-W. Arends, N. E. P. Deutz, and P. B. Soeters.** Glutamine and the preservation of gut integrity. *Lancet* 334: 1363–1365, 1993.
37. **Vinnars, E., J. Bergström, and P. Fürst.** Influence of the post operative state on the intracellular free amino acids in human muscle tissue. *Ann. Surg.* 182: 665–671, 1975.
38. **Welle, S., C. Thornton, R. Jozefowicz, and M. Statt.** Myofibrillar protein synthesis in young and old men. *Am. J. Physiol.* 264 (*Endocrinol. Metab.* 27): E693–E698, 1993.
39. **Wernerman, J., R. Brandt, T. Strandell, L. G. Allgen, and E. Vinnars.** The effect of stress hormones on the interorgan flux of amino acids and on the concentration of free amino acids in skeletal muscle. *Clin. Nutr.* 4: 207–216, 1985.
40. **Wernerman, J., A. von der Decken, and E. Vinnars.** Protein synthesis in skeletal muscle in relation to nitrogen balance after abdominal surgery: effect of parenteral nutrition. *J. Parenter. Enteral Nutr.* 10: 578–582, 1986.
41. **Wolfe, R. R.** *Radioactive and Stable Isotope Tracers in Biomedicine: Principles and Practice of Kinetic Analysis.* New York: Wiley-Liss, 1992, p. 419–420.
42. **Zeigler, T. R., L. S. Young, K. Benfell, M. Scheltinga, K. Hortos, R. Bye, F. D. Morrow, D. O. Jacobs, R. J. Smith, J. H. Antin, and D. W. Wilmore.** Clinical and metabolic efficacy of glutamine supplemented parenteral nutrition after bone marrow transplantation. *Ann. Intern. Med.* 116: 821–828, 1992.

Am. J. Physiol. Endocrinol. Metab.
278: E226–E233, 2000.

Effects of glutamine supplementation, GH, and IGF-I on glutamine metabolism in critically ill patients

N. C. JACKSON,[1] P. V. CARROLL,[3] D. L. RUSSELL-JONES,[1]
P. H. SÖNKSEN,[2] D. F. TREACHER,[1] AND A. M. UMPLEBY[1]
[1]*Departments of Diabetes, Endocrinology and Metabolic Medicine and* [2]*Intensive Care, St. Thomas' Hospital, London SE1 7EH; and* [3]*Department of Medicine, Greenwich District Hospital, Greenwich, London SE10 9HE, United Kingdom*

Jackson, N. C., P. V. Carroll, D. L. Russell-Jones, P. H. Sönksen, D. F. Treacher, and A. M. Umpleby. Effects of glutamine supplementation, GH, and IGF-I on glutamine metabolism in critically ill patients. *Am. J. Physiol. Endocrinol. Metab.* 278: E226–E233, 2000.—During critical illness glutamine deficiency may develop. Glutamine supplementation can restore plasma concentration to normal, but the effect on glutamine metabolism is unknown. The use of growth hormone (GH) and insulin-like growth factor I (IGF-I) to prevent protein catabolism in these patients may exacerbate the glutamine deficiency. We have investigated, in critically ill patients, the effects of 72 h of treatment with standard parenteral nutrition (TPN; $n = 6$), TPN supplemented with glutamine (TPNGLN; 0.4 $g \cdot kg^{-1} \cdot day^{-1}$, $n = 6$), or TPNGLN with combined GH (0.2 $IU \cdot kg^{-1} \cdot day^{-1}$) and IGF-I (160 $\mu g \cdot kg^{-1} \cdot day^{-1}$) (TPNGLN+GH/IGF-I; $n = 5$) on glutamine metabolism using [2-^{15}N]glutamine. In patients receiving TPNGLN and TPNGLN+GH/IGF-I, plasma glutamine concentration was increased (338 ± 22 vs. 461 ± 24 μmol/l, $P < 0.001$, and 307 ± 65 vs. 524 ± 71 μmol/l, $P < 0.05$, respectively) and glutamine uptake was increased (5.2 ± 0.5 vs. 7.4 ± 0.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < 0.05$ and 5.2 ± 1.1 vs. 7.6 ± 0.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < 0.05$). Glutamine production and metabolic clearance rates were not altered by the three treatments. These results suggest that there is an increased requirement for glutamine in critically ill patients. Combined GH/IGF-I treatment with TPNGLN did not have adverse effects on glutamine metabolism.

stable isotopes; nutritional support; catabolism; postoperative care

DURING CRITICAL ILLNESS amino acids are mobilized from peripheral tissue, such as muscle, and are used by organs in the splanchnic area for gluconeogenesis, oxidation, ureagenesis, protein synthesis, and also as substrates for the immune system and wound healing (9). This is thought to lead to the wasting of lean body mass characteristic of critical illness, which often persists despite nutritional support. Because maintenance of body protein stores and the integrity of the gut mucosa may have an impact on morbidity and mortality, several strategies are currently being investigated to prevent the loss of lean tissue. These include the use of specialized nutrition and the use of anabolic hormones, such as growth hormone (GH) and insulin-like growth factor I (IGF-I).

Glutamine is the most abundant amino acid in the body and is traditionally classified as a nonessential amino acid because it is synthesized endogenously (2). However, marked decreases in free glutamine concentrations have been reported in a variety of catabolic states (1, 37). This suggests that during serious illness a deficiency in glutamine availability may develop and has led to the idea that glutamine is a conditionally essential amino acid (22).

At present, glutamine is not routinely added to parenteral nutrition solutions, but recent clinical trials suggest that glutamine supplementation improves both nitrogen balance and gut mucosal integrity and decreases the number of infections, length of hospital stay, and 6-mo mortality in critically ill patients (13, 36, 41). In a previous study we showed that, despite a fall in plasma glutamine concentration and an increase in glutamine clearance, plasma glutamine flux was unchanged in critically ill patients compared with matched healthy controls (18). Despite the current clinical interest in the potential benefits of glutamine supplementation, there have been no tracer studies investigating the effects of glutamine supplementation on glutamine metabolism in critically ill patients.

The availability of recombinant human GH (rhGH) and IGF-I (rhIGF-I) has led to considerable interest in their use, either alone or in combination, to reduce protein catabolism in a variety of catabolic states. However, evidence from recent multicenter trials has linked GH treatment with increased mortality in intensive care unit (ICU) patients (34). In these trials the patients did not receive routine glutamine supplementation. Because during periods of illness glutamine is mobilized from protein stores (sketetal muscle), an increase in protein anabolism may exacerbate the glutamine depletion seen in these patients. It is possible that this may have contributed to the increased mortality observed in these trials.

The aim of the present study was to use [2-^{15}N]glutamine to investigate the effects of standard total parenteral nutrition (TPN) and parenteral nutrition supplemented with glutamine (TPNGLN) on whole body glutamine metabolism in critically ill patients. We also

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

studied the addition of combined treatment with GH plus IGF-I and TPNGLN (TPNGLN+GH/IGF-I) on glutamine metabolism.

METHODS

Materials. L-[2-^{15}N]glutamine [99 atom percent (AP)] and L-[1-^{13}C]leucine (99 AP) were purchased from Tracer Technologies (Somerville, MA). Sterile solutions of the tracers were prepared in 0.9% saline with an aseptic technique by the Pharmacy Department of Guy's and St Thomas' Hospital (London, UK). The standard TPN consisted of 50% dextrose, Intralipid 20% (Kabivitrum, Stockholm, Sweden), and a mixed amino acid solution (Vamin 14; Pharmacia & Upjohn, Milton Keynes, UK). L-Glutamine solution was purchased from Oxford Nutrition (Oxford, UK) and was stored at −20°C until use. GH and IGF-I were supplied by Pharmacia & Upjohn.

Subjects. Nineteen severely ill patients in the ICU of St Thomas' Hospital were initially studied. Two patients died before completion of the second study; thus data are presented only from the 17 patients who survived to complete both studies. However, as indicated in Table 1, one patient later died in the ICU. The patients were all newly admitted to the ICU, and the majority had undergone emergency abdominal surgery. All required mechanical ventilation and intravenous nutritional support. Further details of their clinical and metabolic characteristics are summarized in Table 1. The severity of illness was evaluated on the day of the study by use of the APACHE II and TISS score systems (5, 21).

The protocol was approved by the Ethics Committee, Guy's and St. Thomas' National Health Service Trust. Written informed consent was obtained from relatives of the patients.

Study protocol. All of the patients were fasted for ≥12 h before the start of the first study. Indwelling arterial and central venous lines were used for blood sampling and for the tracer infusion, respectively. After baseline sampling, a priming bolus of [1-^{13}C]leucine (1 mg/kg) was injected, and 4-h constant infusions of [2-^{15}N]glutamine (2.5 mg·kg^{-1}·h^{-1}) and [1-^{13}C]leucine (1 mg·kg^{-1}·h^{-1}) were started. Blood samples were taken at 210, 215, 220, 225, 230, and 240 min for steady-state measurement of plasma glutamine and α-ketoisocaproic acid (α-KIC) enrichment and glutamine concentration. Samples were also taken at steady state for the measurement of routine biochemistry, metabolite, and hormone levels [glucose, insulin, IGF-I, IGF-binding protein-1 (IGFBP-1), cortisol, glucagon, thyroid hormones, and amino acids]. For glutamine analysis, 0.5-ml lithium heparin plasma aliquots were mixed with 100 μl internal standard (100 nmol L-[U-$^{13}C_5$]glutamine). All samples were stored at −70°C until analysis.

At the end of the baseline study, patients were randomized to receive either standard TPN, TPN with additional intrave-

Table 1. *Clinical and metabolic characteristics of patients*

Patient No.	Gender	Age, yr	Weight, kg	Height, cm	BMI, kg/m^2	APACHE II	TISS	Plasma Albumin, g/dl	Plasma CRP, mg/dl	Main Diagnosis	28 Day Mortality
						TPN					
1	F	68	64	171	22	15	34	34	82	Bowel Resection	−
2	F	55	42	158	17	20	47	28	191	Bowel Resection	−
3	M	76	74	182	22	23	49	27	68	GI Bleed/laparotomy	−
4	M	74	66	175	21	20	48	18	217	AAA	−
5	F	69	61	162	23	18	42	25	185	AAA	−
6	M	73	63	170	22	19	49	22	212	AAA	−
Mean ± SE		69 ± 3	62 ± 4	170 ± 4	21 ± 1	19 ± 1	45 ± 2				
						TPNGLN					
1	M	72	82	187	23	15	37	23	161	GI Obstruction	−
2	F	65	75	170	26	9	50	19	557	Bowel Resection	−
3	M	75	104	183	31	24	47	27	203	AAA	−
4	F	75	85	168	30	19	38	23	329	Bowel Obstruction	−
5	M	59	98	181	30	14	47	20	247	Bowel Resection	−
6	F	32	59	170	20	18	62	17	145	Bowel Resection	−
Mean ± SE		63 ± 7	84 ± 7	177 ± 3	24 ± 3	17 ± 2	46 ± 3				
						TPNGLN + GH/IGF-I					
1	M	65	65	182	20	19	50	15	378	Bowel Resection	−
2	F	54	61	171	21	20	52	11	105	Bowel Resection	+
3	M	50	60	179	19	9	38	26	38	GI Obstruction	−
4	M	62	115	179	36	16	42	20	249	Cardiac Arrest	−
5	M	78	76	175	25	14	47		361	AAA	−
Mean ± SE		62 ± 5	75 ± 10	177 ± 2	24 ± 3	16 ± 2	46 ± 3				

TPN, total parenteral nutrition; TPNGLN, TPN + supplemental Gln; TPNGLN + GH/IGF-I, TPNGLN + growth hormone and insulin-like growth factor I; BMI, body mass index; GI, gastrointestinal; AAA, abdominal aortic aneurysm; CRP, C-reactive protein; APACHE II and TISS, scoring systems for disease severity classification.

nous glutamine (TPNGLN: 0.4 $g \cdot kg^{-1} \cdot day^{-1}$), or TPNGLN with rhGH (0.2 $IU \cdot kg^{-1} \cdot day^{-1}$) and rhIGF-I (160 $\mu g \cdot kg^{-1} \cdot day^{-1}$). The patients randomized to TPNGLN received additional nitrogen in the form of the glutamine infusion (~0.06 g $N \cdot kg^{-1} \cdot day^{-1}$). The nutritional support was not made isonitrogenous because this could have limited the availability of other amino acids. A continuous insulin infusion (Actrapid; Novo Nordisk, Copenhagen, Denmark) was provided if required to maintain plasma glucose concentration at or below 7 mmol/l. The GH was administered as a single subcutaneous injection; the dose of IGF-I was split into two equal twice daily subcutaneous injections.

After 72 h of treatment, a second turnover study was performed using the same tracers and infusion rates as the initial study. In this second study the patients were not fasted, as nutritional support was continued throughout the protocol (Fig. 1). The patients were all sedated and mechanically ventilated (Servo 900; Siemens, Berlin, Germany) throughout both studies.

Experimental methods. The isotopic enrichment and concentration of glutamine were determined from the *tert*-butyldimethylsilyl derivative by use of a method modified from Wolfe (40). Glutamine concentration was determined by reverse isotope dilution using L-[U-$^{13}C_5$]glutamine (Bioquote, North Yorkshire, UK) as the internal standard. Analysis by gas chromatography-mass spectrometry (GC-MS; MSD 5971A, Hewlett-Packard, Berkshire, UK) used electron impact ionization with selected ion monitoring of the [M-butyl]$^+$ ions at mass-to-charge ratios (*m/z*) 431, 432, and 436. The isotopic enrichment of α-KIC was measured as the quinoxalinol-*tert*-butyldimethylsilyl derivative by use of a method modified from Ford and co-workers (8). GC-MS analysis used electron impact ionization with selected ion monitoring of the [M-butyl]$^+$ ions at *m/z* 259 and 260. Plasma glucose concentrations were measured on a model 23AM glucose analyzer (YSI, Hampshire, UK). Serum insulin concentrations were measured by an in-house double-antibody RIA (32). Total IGF-I was measured by RIA after acid ethanol extraction. Total IGFBP-1 was measured by a coated-tube immunoradiometric assay (Diagnostic Systems Laboratories, Webster, TX). Cortisol was measured by ELISA using the Enzymun-Test cortisol kit (Boehringer Mannheim, Sussex, UK). Glucagon was measured using a commercially available RIA kit (Linco Research, St Louis, MO). Free thyroid hormones were measured by a competitive immunoassay using chemiluminescence (Chiron Diagnostics, Essex, UK). Plasma amino acid concentrations were measured on an Alpha II+ automated amino acid analyzer (Pharmacia, Hertfordshire, UK). Plasma albumin and C-reactive protein were measured using an automated method (Kodak 250, Ortho Clinical Diagnostics, Amersham, UK).

Calculations. Measurements of glutamine metabolism were calculated using standard isotope dilution equations. Glutamine appearance rate (Ra_{Gln}; $\mu mol \cdot min^{-1} \cdot kg^{-1}$) was calculated as $Ra_{Gln} = F[1/(APE_{Gln} \cdot 0.01) - 1]$, where F is the isotope infusion rate ($\mu mol \cdot min^{-1} \cdot kg^{-1}$), and APE_{Gln} is the plasma glutamine enrichment. In the postabsorptive state, the endogenous glutamine rate of appearance in plasma (Endo Ra_{Gln}) is equal to the calculated Ra_{Gln}. At steady state, the rate of disappearance of glutamine from plasma (glutamine uptake: Rd_{Gln}) was assumed to be equal to Ra_{Gln}. Leucine appearance rate was calculated using an analogous equation but with use of the plasma enrichment of α-KIC as a measure of intracellular leucine enrichment (27). Glutamine metabolic clearance rate (MCR; $ml \cdot min^{-1} \cdot kg^{-1}$) was calculated as $MCR = Rd_{Gln}/[Gln]$, where [Gln] is the steady-state plasma glutamine concentration (μmol/ml).

Because glutamine is a nonessential amino acid in the fasting state, Ra_{Gln} is derived from both protein breakdown (B_{Gln}) and de novo glutamine synthesis (D_{Gln}). B_{Gln} was estimated as 0.78 × Ra_{Leu} (7), and D_{Gln} was calculated as $D_{Gln} = Ra_{Gln} - B_{Gln}$ (15).

Nutritional support was maintained throughout the second study. Because the patients were not in the postabsorptive state, the equations given above were corrected for the known exogenous rates of leucine and glutamine infusion. In all three treatment groups, the exogenous rate of infusion of leucine (I_{Leu}) was calculated from the TPN regimen, and the endogenous leucine rate of appearance in plasma from protein breakdown (Endo Ra_{Leu}) was estimated as Endo $Ra_{Leu} = Ra_{Leu} - I_{Leu}$. In the group of patients receiving TPN without glutamine supplementation, B_{Gln} was estimated using Endo Ra_{Leu}, and D_{Gln} was calculated as shown above.

For the two groups of patients receiving TPNGLN, the exogenous rate of glutamine infusion (I_{Gln}) was calculated, and the endogenous glutamine rate of appearance in plasma (Endo Ra_{Gln}) was estimated as Endo $Ra_{Gln} = Ra_{Gln} - I_{Gln}$. B_{Gln} was estimated using Endo Ra_{Leu}, and D_{Gln} was calculated using Endo Ra_{Gln}.

Statistics. All data are presented as means ± SE. "Steady state" for plasma glutamine and α-KIC enrichments was confirmed as an insignificant correlation with time ($P > 0.05$) by use of repeated-measures ANOVA (NCSS 6.0, Dr. J. Hintze, Kaysville, UT). Comparisons between groups were

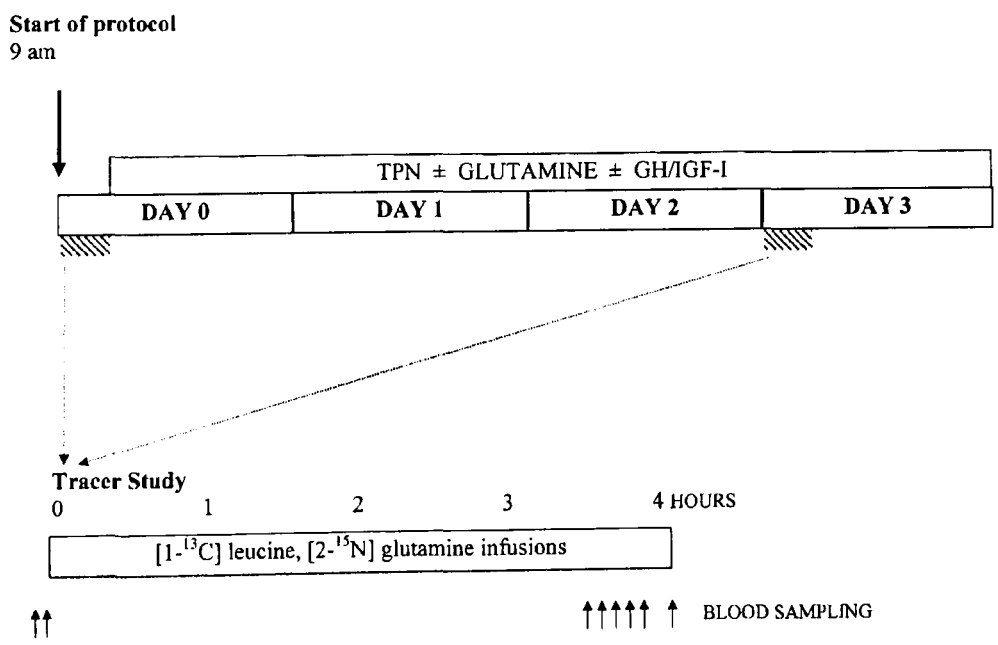


Fig. 1. Schematic representation of study protocol. All patients were initially studied in the fasting state and were then randomized to receive either total parenteral nutrition (TPN, $n = 6$), TPN + Gln (TPNGLN, $n = 6$), or TPNGLN + growth hormone (GH) + insulin-like growth factor (IGF-I) (TPNGLN+GH/IGF-I, $n = 5$). After 72 h of nutritional ± hormonal treatment, a second tracer study was performed during which nutritional support was continued. Hatched areas represent tracer studies. Infusion protocol is shown at bottom of diagram.

made using ANOVA, and comparisons between *study 1* and *study 2* were made by standard two-tailed paired *t*-tests. The insulin, IGFBP-1, cortisol, and glucagon data were log transformed before analysis.

RESULTS

Table 1 shows the details of the 17 ICU patients studied. There were no significant differences in age, weight, height, or body mass index among the three treatment groups. The severity of illness is indicated by the APACHE II and TISS scores, which identify the patients as being severely ill and dependent on cardiorespiratory and nutritional support (5, 21). There were no significant differences in the APACHE II and TISS scores among the three groups of ICU patients at the start of the study. None of the patients was acidemic, and there were no differences in the arterial pH values among the patient groups in *study 1* (TPN, 7.43 ± 0.03; TPNGLN, 7.44 ± 0.02; TPNGLN+GH/IGF-I, 7.39 ± 0.02). In addition, bicarbonate concentrations indicated no depletion of alkaline reserves (TPN, 26 ± 2 mmol/l; TPNGLN, 26 ± 1.5 mmol/l; TPNGLN+GH/IGF-I, 25 ± 2.3 mmol/l). There were no significant changes in arterial blood gas analysis between *study 1* and *study 2*. Plasma urea concentrations were elevated in all groups in *study 1*, indicating a high rate of protein catabolism (TPN, 13.0 ± 2.3 mmol/l; TPNGLN, 12.9 ± 2.5 mmol/l; TPNGLN+GH/IGF-I, 9.3 ± 1.4 mmol/l; normal reference range 4–7 mmol/l).

The glucose and insulin data are shown in Fig. 2. There was a trend toward increased plasma glucose levels in the second studies for all three treatment groups. However, this increase only reached significance in the TPN and TPNGLN+GH/IGF-I groups ($P < 0.05$). Plasma insulin levels increased in *study 2* for all three treatment groups (TPN, $P < 0.01$; TPNLGLN, $P < 0.05$); however, this failed to reach significance in the TPNGLN+GH/IGF-I group ($P = 0.13$). Insulin infusion rates in *study 2* were 1.0 ± 0.4 U/h in the TPN ($n = 4$) and 1.5 ± 0.6 U/h in the TPNGLN ($n = 4$) patient groups. In the TPNGLN+GH group only one patient received 2 U/h insulin during *study 2*. The measured total IGF-I concentrations were similar in all three groups in *study 1* (TPN, 13.6 ± 1.8 nmol/l; TPNGLN, 12.1 ± 0.9 nmol/l; TPNGLN+GH/IGF-I, 10.3 ± 0.8 nmol/l), and these values were lower than those reported from healthy control subjects of a similar age (16.5 ± 1.7 nmol/l; see Ref. 18). Total IGF-I was unchanged after both TPN and TPNGLN; however, as expected, there was a significant increase in *study 2* (48.1 ± 9.1 nmol/l) in the TPNGLN+GH/IGF-I group ($P < 0.05$). IGFBP-1 was significantly decreased in *study 2* in the TPN group (231 ± 79 vs. 39 ± 12 ng/ml, $P < 0.05$); however, the changes in the TPNGLN (87 ± 35 vs. 132 ± 86 ng/ml) and TPNGLN+GH/IGF-I (134 ± 59 vs. 58 ± 15 ng/ml) groups were not significant. There were no significant changes in cortisol, free thyroxine, free triiodothyronine, or glucagon levels between *studies 1* and *2* for the three treatment groups.

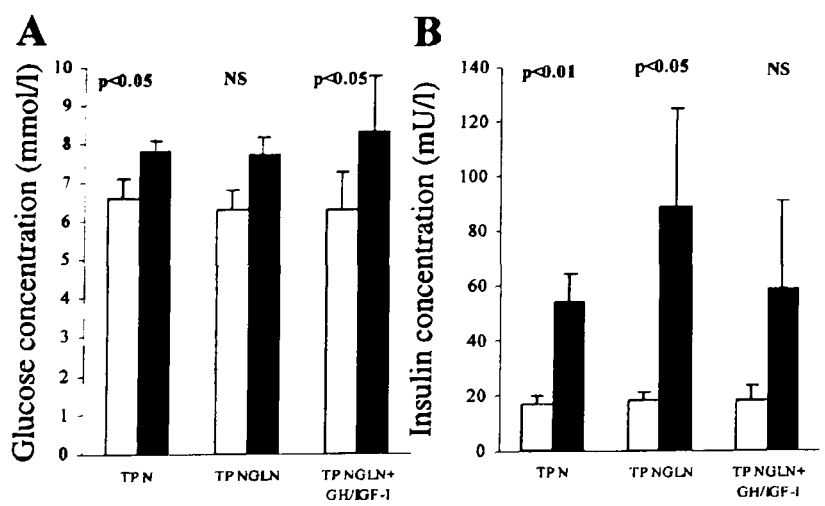


Fig. 2. Glucose (*A*) and insulin concentrations (*B*) in *study 1* (open bars) and *study 2* (filled bars). Values are means ± SE.

Plasma amino acid profiles are shown in Table 2. In the group receiving TPN there were significant increases in plasma serine ($P < 0.05$), glycine ($P < 0.05$), alanine ($P < 0.05$), methionine ($P < 0.01$), and ornithine ($P < 0.05$) levels in *study 2*. However, the increase in total amino acids did not reach significance ($P = 0.07$). Plasma serine ($P < 0.05$), glutamate ($P < 0.05$), glutamine ($P < 0.001$), glycine ($P < 0.05$), alanine ($P < 0.001$), methionine ($P < 0.05$), histidine ($P < 0.01$), and total amino acids ($P < 0.01$) were all significantly increased in the second study in the patients receiving TPNGLN. In the group receiving TPNGLN+GH/IGF-I, the plasma profiles were similar in *studies 1* and *2*, apart from increases in glutamine ($P < 0.05$) and phenylalanine concentrations ($P < 0.05$).

Figure 3 shows the plasma glutamine enrichments and concentrations from *studies 1* and *2* for the TPNGLN patient group during the final 30 min of tracer infusion, indicating that steady state was achieved. Similar glutamine enrichment and concentration steady states were achieved for the other two patient groups (data not shown). The glutamine metabolic data are summarized in Fig. 4. Glutamine production rate and MCR were not affected by any of the three treatments. Glutamine uptake was significantly increased in the second study in both the TPNGLN (5.2 ± 0.5 vs. 7.4 ± 0.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < 0.05$) and TPNGLN+GH/IGF-I (5.2 ± 1.1 vs. 7.6 ± 0.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < 0.05$) groups but was unchanged in the TPN group.

B_{Gln} was significantly decreased in *study 2* in the TPN group (2.5 ± 0.2 vs. 2.1 ± 0.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < 0.01$), and although a similar trend was observed in the TPNGLN group, this failed to reach significance (2.0 ± 0.1 vs. 1.7 ± 0.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P = 0.08$). When expressed as a percentage of Endo Ra_{Gln}, protein-derived glutamine release was decreased after treatment with TPN (44 ± 4 vs. 40 ± 3%, $P < 0.05$) and TPNGLN (40 ± 3 vs. 32 ± 3%, $P < 0.05$; Fig. 5). In the TPNGLN+GH/IGF-I group there was no significant change in B_{Gln} (2.1 ± 0.3 vs. 1.9 ± 0.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). The decrease in the percentage of the glutamine Ra derived from proteolysis also failed to reach significance in this group (46 ± 6 vs. 35 ± 4%, $P = 0.08$; Fig. 5).

D_{Gln} was not significantly altered by treatment with TPN (3.2 ± 0.5 vs. 3.3 ± 0.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$),

Table 2. *Plasma amino acid profiles*

	TPN		TPNGLN		TPNGLN + GH/IGF-I	
	Study 1	*Study 2*	*Study 1*	*Study 2*	*Study 1*	*Study 2*
Aspartate	34 ± 10	38 ± 9	14 ± 6	20 ± 6	18 ± 5	28 ± 12
Threonine	49 ± 8	91 ± 15	57 ± 6	73 ± 8	57 ± 7	67 ± 10
Serine	46 ± 4	105 ± 16*	54 ± 6	84 ± 13*	57 ± 9	73 ± 14
Glutamate	30 ± 8	94 ± 29	36 ± 7	61 ± 14*	43 ± 26	48 ± 10
Glutamine	376 ± 34	426 ± 58	338 ± 22	461 ± 24‡	307 ± 65	524 ± 71*
Glycine	153 ± 29	318 ± 68*	145 ± 13	220 ± 23*	162 ± 29	265 ± 63
Alanine	200 ± 35	414 ± 72*	170 ± 20	278 ± 26‡	183 ± 42	286 ± 83
Valine	191 ± 29	222 ± 28	158 ± 13	174 ± 17	148 ± 24	165 ± 23
Cystine	50 ± 9	70 ± 17	55 ± 14	50 ± 4	37 ± 5	49 ± 8
Methionine	11 ± 2	27 ± 2†	17 ± 2	31 ± 6*	14 ± 2	22 ± 6
Isoleucine	36 ± 8	32 ± 4	41 ± 6	42 ± 8	27 ± 4	30 ± 3
Leucine	116 ± 19	112 ± 18	98 ± 8	86 ± 9	84 ± 15	79 ± 12
Tyrosine	41 ± 9	38 ± 7	49 ± 7	36 ± 12	62 ± 22	36 ± 4
Phenylalanine	80 ± 8	105 ± 9	82 ± 10	105 ± 20	65 ± 7	82 ± 8*
Ornithine	32 ± 3	95 ± 22*	40 ± 5	72 ± 16	35 ± 9	36 ± 7
Lysine	99 ± 12	144 ± 18	105 ± 12	125 ± 11	115 ± 15	113 ± 16
Histidine	41 ± 4	52 ± 4	38 ± 5	61 ± 8†	46 ± 7	54 ± 8
Total	1,546 ± 149	2,324 ± 309	1,463 ± 94	1,967 ± 108†	1,434 ± 161	1,894 ± 270
BCAA	337 ± 54	365 ± 49	290 ± 29	302 ± 31	260 ± 40	274 ± 33

Values are means ± SE expressed in μmol/l. BCAA, branched-chain amino acids. * $P < 0.05$ *study 1* vs. *study 2*; † $P < 0.01$ *study 1* vs. *study 2*; ‡ $P < 0.001$ *study 1* vs. *study 2*.

TPNGLN (3.1 ± 0.5 vs. 3.8 ± 0.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$), or TPNGLN+GH/IGF-I (3.0 ± 1.0 vs. 3.9 ± 0.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). However, the percentage of the glutamine Ra arising from D_{Gln} was significantly increased after treatment with TPN (56 ± 4 vs. 60 ± 3%, $P < 0.05$) and TPNGLN (60 ± 3 vs. 68 ± 3%, $P < 0.05$; Fig. 5). Although a similar trend was seen in the TPNGLN+GH/IGF-I group, this failed to reach significance (54 ± 6 vs. 65 ± 4%, $P = 0.08$; Fig. 5).

DISCUSSION

This study demonstrates that supplementation of TPN with glutamine in critically ill patients restores plasma glutamine concentration to nearly normal levels and increases glutamine uptake. A similar increase in plasma glutamine concentration and glutamine uptake was found when combined GH/IGF-I therapy was added to TPN with glutamine supplementation. This suggests that combined GH/IGF-I therapy does not have adverse effects on glutamine metabolism in these patients.

Marked decreases in free glutamine concentrations have been reported in catabolic states associated with protein wasting. To date it has not been established whether this fall in glutamine concentration is a true glutamine deficiency or an alteration in glutamine homeostasis related to severe illness. Several studies have shown that the addition of glutamine or its analogs to TPN improves nitrogen balance and reduces the fall in muscle glutamine concentration and protein synthesis in postoperative patients (3, 14, 30, 33, 39). In the present study, we found that glutamine supplementation to TPN increased plasma glutamine concentration. The patients were fasted for ≥12 h before the first study, but nutritional support was continued throughout the second study. By using an isotopic tracer of glutamine to measure glutamine metabolism, we were

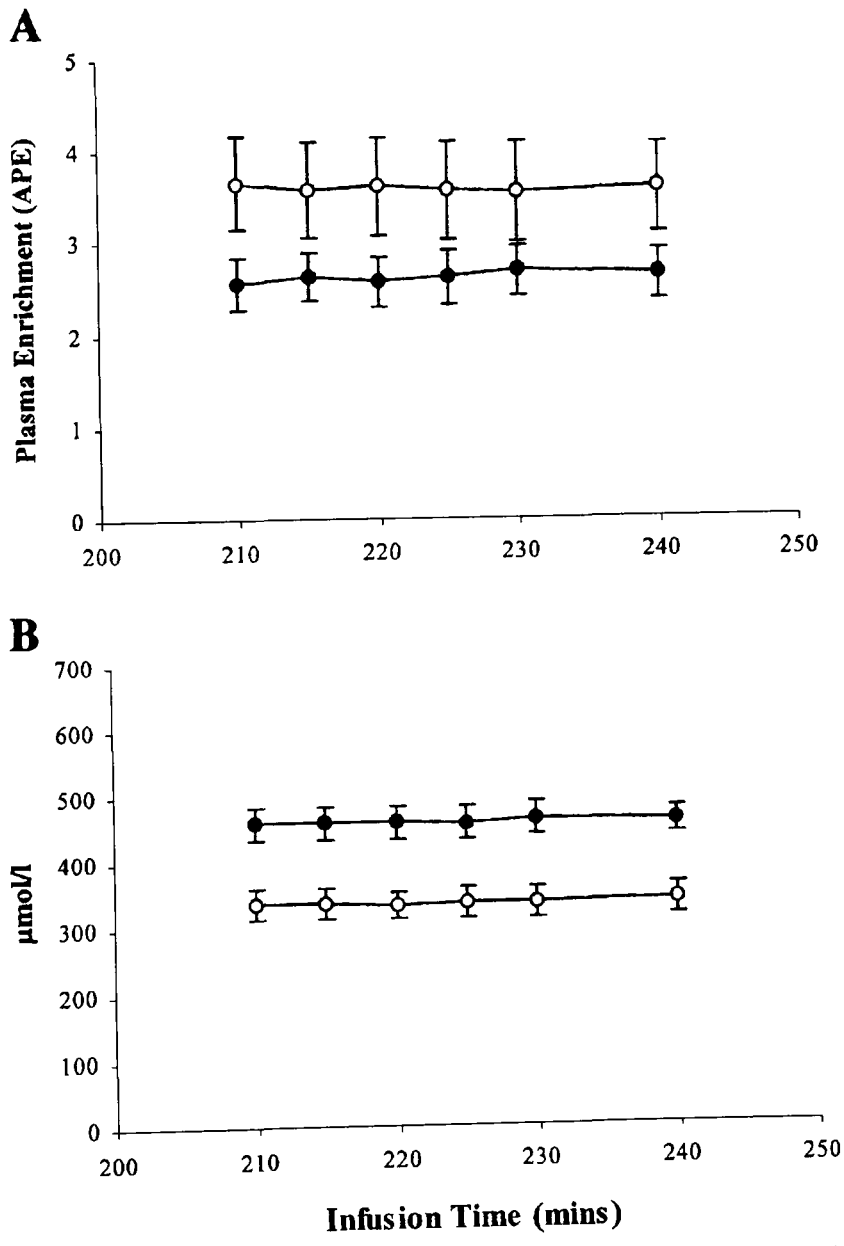


Fig. 3. Plasma glutamine enrichment (atom percent excess, APE; *A*) and concentration (μmol/l, *B*) for total parenteral nutrition + Gln (TPNGLN) patients in *study 1* (○) and *study 2* (●). Values are means ± SE; $n = 6$.

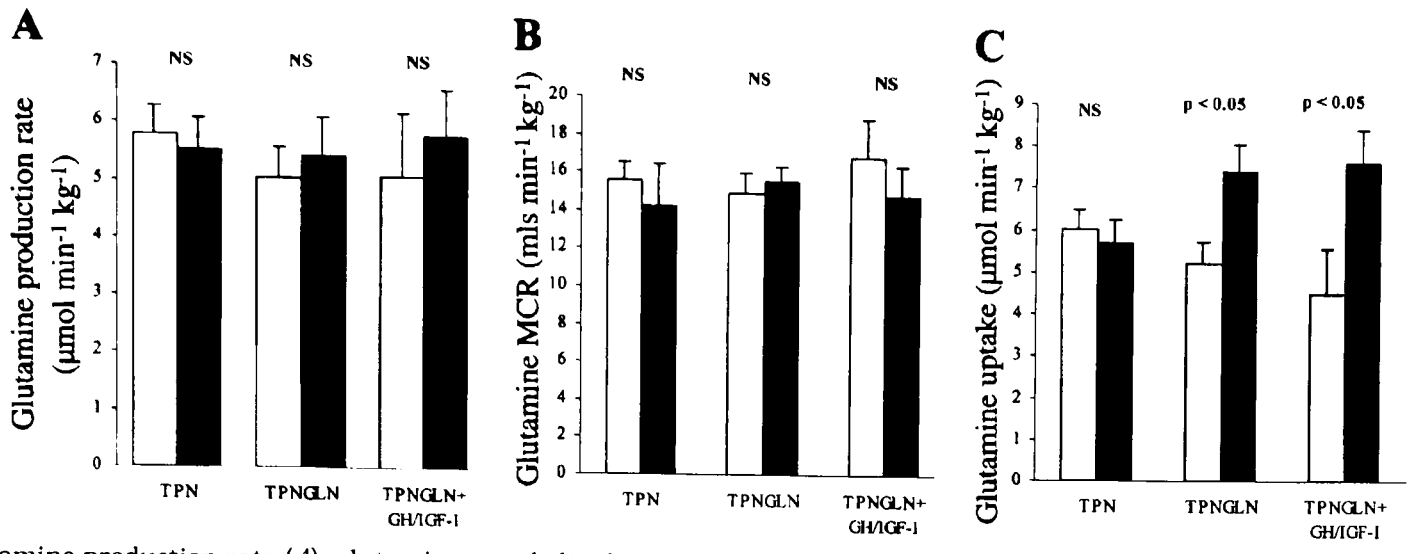


Fig. 4. Glutamine production rate (*A*), glutamine metabolic clearance rate (MCR; *B*), and glutamine uptake (*C*) in *study 1* (open bars) and *study 2* (filled bars). Values are means ± SE.

also able to demonstrate that whole body glutamine uptake was increased. This increased uptake may be for use by tissues with high metabolic demands for glutamine, such as the immune system and the gastrointestinal tract. TPN without glutamine supplementation failed to normalize glutamine concentration and had no effect on glutamine uptake, suggesting that glutamine supplementation may be important to meet the increased metabolic demands of glutamine-requiring tissues in the catabolic state.

In a previous study we showed that, despite a marked decrease in plasma glutamine concentration, plasma glutamine flux was unchanged in critically ill patients compared with matched healthy controls. We also found that glutamine MCR was increased in these patients, suggesting that the primary mechanism for the reduced concentration may be an increased efficiency of glutamine transport (18). In the present study when exogenous glutamine was provided, with or without GH/IGF-I treatment, glutamine MCR remained elevated. The increased glutamine uptake would thus have been facilitated by the increased efficiency of glutamine transport.

Acute glutamine depletion induced by phenylbutyrate in healthy adults has also been shown not to affect the glutamine appearance rate (7). However, Gore and Jahoor (12) reported an increased glutamine flux (60%) in burns patients compared with controls. In the present study there was a decrease in the proportion of endogenous glutamine production derived from protein breakdown when the patients were receiving nutritional support, although this decrease did not achieve significance in the TPNGLN+GH/IGF-I-treated patients.

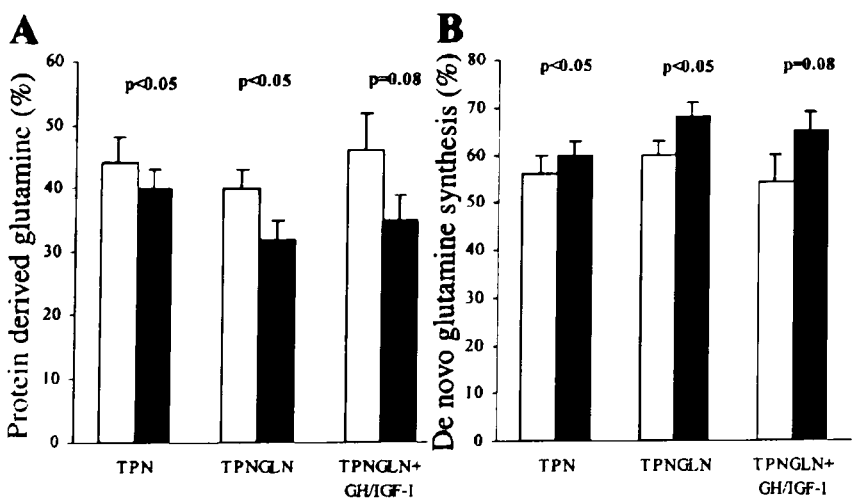


Fig. 5. Percentage of endogenous glutamine appearance rate derived from protein breakdown (*A*) and de novo synthesis (*B*) in *study 1* (open bars) and *study 2* (filled bars). Values are means ± SE.

Exogenous insulin was given when necessary to maintain the patients' blood glucose at or below 7 mmol/l, a standard procedure in the ICU at St Thomas' Hospital. This treatment contributed to the observed increase in plasma insulin concentration in the TPN and TPNGLN groups. The increase in insulin concentration was not statistically significant in the TPNGLN+GH/IGF-I group; this may be related to the fact that only one patient received exogenous insulin in this group. Because insulin plays a central role in protein metabolism by reducing protein breakdown (10), this may account for some of the decrease in glutamine production derived from protein breakdown in the TPN and TPNGLN groups. Similarly, the insulin deficiency of type 1 diabetes mellitus has been shown to increase the proportion of the glutamine appearance from protein breakdown (6). The fact that glutamine production rate was unchanged in the present study suggests that there was a shift to de novo synthesis of glutamine from the exogenous supply of amino acids.

Many of the early studies on the effects of glutamine supplementation assessed nitrogen balance and protein synthesis using ribosomal analysis. The improvement in nitrogen balance with glutamine supplementation led to the assumption that some of the benefits associated with glutamine supplementation were mediated through alterations in protein metabolism and the preservation of muscle mass. In addition, experiments using animal models had indicated a positive relationship between protein synthesis and glutamine concentration (20, 26). More recently, enteral glutamine infusions in healthy adults have been shown to increase protein synthesis (16). However, there is no direct evidence from studies in humans that increased muscle glutamine concentration enhances protein synthesis in

catabolic states. Whole body protein metabolism in critically ill patients was not affected by glutamine supplementation of enteral nutrition (24). Interpretation of the improvement in nitrogen balance needs caution, as a significant proportion of this balance may be due to replenishment of the muscle glutamine pool rather than synthesis of glutamine-containing proteins (38).

The benefits of glutamine supplementation may be mediated through other tissues and pathways rather than via a direct effect on muscle and protein. Glutamine supplementation has been reported to have a beneficial effect on intestinal function and the intestinal mucosa in patients receiving intravenous nutrition (35, 36). A decrease in the number of infections and reduced hospital stay and costs have been reported in bone marrow transplant patients receiving TPN supplemented with glutamine (25, 41), suggesting possible effects on immune function, and glutamine is recognized to be a substrate for immunocytes (29). An improvement in 6-mo survival has also been reported in critically ill patients receiving glutamine-supplemented TPN, but the mechanism(s) for this improvement remain unclear (13).

The loss of lean tissue in the critically ill has led to the investigation of treatments to preserve lean tissue by use of various protein anabolic agents. GH treatment has been shown to improve nitrogen balance, increase protein synthesis, and reduce leucine oxidation in parenterally fed catabolic patients (4, 19). The demonstration that GH had protein anabolic effects led to two European multicenter trials being conducted in ICU patients. However, rather than improving mortality, as was expected, exogenous GH resulted in a significant increase in mortality (42 vs. 18%) (34). These multicenter studies differed from the present study in that the patients were given a variety of nutritional supports excluding glutamine supplementation. In addition, in the present study the GH treatment was given only in combination with IGF-I therapy.

An increase in protein anabolism as a result of GH and IGF-I treatment may result in a decrease in glutamine availability, exacerbating the glutamine depletion seen in the critically ill. Supporting this possibility is the finding that GH reduces skeletal muscle glutamine release in catabolic states (28). It is therefore possible that decreased glutamine availability may have contributed to the increased mortality in the multicenter ICU trials. Because IGF-I has protein anabolic effects, there is also interest in the use of this hormone in the treatment of catabolic patients (11, 23). In normal subjects, combined GH/IGF-I has been shown to have a synergistic effect on protein synthesis (31). Thus, in the present study, the effect of combined treatment with GH/IGF-I was investigated. These results show that combined GH and IGF-I treatment did not decrease glutamine production rate in these critically ill patients. The increase in glutamine concentration and glutamine uptake was similar to that achieved in the TPNGLN group, suggesting that this treatment did not have adverse effects on glutamine metabolism when nutritional support was supplemented with glutamine.

Many of the clinical studies of glutamine supplementation have studied patients undergoing elective surgery. These are homogenous groups of patients undergoing standard operative traumas, and these subjects are generally less severely ill than patients admitted to the ICU. The patients for the present study were recruited in the ICU from those for whom the clinical decision had been made to use parenteral nutrition. We chose to study these patients because they represent the group in which there is considerable clinical interest in the potential benefits of glutamine supplementation, but they are a very heterogeneous group, as can be seen from the patient details in Table 1. We had originally intended to study 12 patients in each group, but unfortunately the study was terminated because of the withdrawal of GH from use in ICUs after the mortality outcome of the GH multicenter trial. Some of the trends between the studies, for example the changes in insulin and amino acid concentrations, might have reached significance if more patients had been studied.

We have previously shown in the critically ill that glutamine uptake is maintained, despite low glutamine concentrations, by an increase in the efficiency of glutamine transport as demonstrated by the increase in glutamine MCR. This is probably due to the increased metabolic demand for glutamine by tissues such as the immune system and the gastrointestinal tract in the catabolic state. In the present study we have shown that, after glutamine supplementation, glutamine concentration was restored to normal but glutamine uptake was increased. The fact that glutamine MCR remained elevated suggests that an increased efficiency of glutamine transport facilitates the increased uptake. The addition of combined GH/IGF-I therapy to nutritional support with glutamine supplementation did not affect the increase in whole body glutamine uptake or the restoration of plasma glutamine concentration, suggesting that in the presence of glutamine supplementation, this hormone treatment did not have adverse effects on glutamine metabolism.

We are grateful to the nurses and staff of Mead Ward (ICU), St. Thomas' Hospital, for their patience and support. We are also grateful to Pharmacia & Upjohn for supplying the GH and IGF-I. We acknowledge the assistance of Dr. F. Shojaee-Moradie, A. Fofanah, and the Department of Chemical Pathology for sample analysis. We also thank P. Forsey, of the Hospital Pharmacy, for preparation of the tracer solutions.

Address for reprint requests and other correspondence: N. C. Jackson, Dept. of Diabetes, Endocrinology and Metabolic Medicine, 4th Floor North Wing, St. Thomas' Hospital, London SE1 7EH, UK.

Received 30 March 1999; accepted in final form 1 October 1999.

REFERENCES

1. **Askanazi, J., Y. A. Carpentier, C. B. Michelsen, D. H. Elwyn, P. Furst, L. R. Kantrowitz, F. E. Gump, and M. J. Kinney.** Muscle and plasma amino acids following injury: influence of intercurrent infection. *Ann. Surg.* 192: 78–85, 1980.
2. **Bergström, J., P. Fürst, L.-O. Norée, and E. Vinnars.** Intracellular free amino acid concentration in human muscle tissue. *J. Appl. Physiol.* 36: 693–697, 1974.
3. **Blomqvist, B. I., F. Hammerqvist, A. von der Decken, and J. Wernerman.** Glutamine and ketoglutarate prevent the decrease

in muscle free glutamine concentration and influence protein synthesis after hip replacement. *Metabolism* 44: 1215–1222, 1995.

4. **Carli, F., J. D. Webster, and D. Halliday.** Growth hormone modulates amino acid oxidation in the surgical patient: leucine kinetics during the fasted and fed state using moderate nitrogenous and caloric diet and recombinant human growth hormone. *Metabolism* 46: 23–28, 1997.
5. **Cullen, D. J., J. M. Civetta, B. A. Briggs, and L. C. Ferrara.** Therapeutic intervention scoring system: a method for quantitative comparison of patient care. *Crit. Care Med.* 2: 57–60, 1974.
6. **Darmaun, D., M. Rongier, J. Koziet, and J.-J. Robert.** Glutamine nitrogen kinetics in insulin-dependent diabetic humans. *Am. J. Physiol. Endocrinol. Metab.* 261: E713–E718, 1991.
7. **Darmaun, D., S. Welch, A. Rini, B. K. Sager, A. Altomare, and M. W. Haymond.** Phenylbutyrate-induced glutamine depletion in humans: effect on leucine metabolism. *Am. J. Physiol. Endocrinol. Metab.* 274: E801–E807, 1998.
8. **Ford, G. C., K. N. Cheng, and D. Halliday.** The analysis of (1-^{13}C)leucine and (^{13}C)KIC in plasma by capillary gas chromatography mass spectrometry in protein turnover studies. *Biomed. Mass Spectrom.* 12: 432–436, 1985.
9. **Gamrin, L., P. Essen, A. M. Forsberg, E. Hultman, and J. Wernerman.** A descriptive study of skeletal muscle metabolism in critically ill patients: free amino acids, energy rich phosphates, protein, nucleic acids, fat, water and electrolytes. *Crit. Care Med.* 24: 575–583, 1996.
10. **Gelfand, R. A., and E. J. Barrett.** Effect of physiologic hyperinsulinaemia on skeletal muscle protein synthesis and breakdown in man. *J. Clin. Invest.* 80: 1–6, 1987.
11. **Goeters, C., N. Mertes, J. Tacke, U. Bolder, M. Kuhman, P. Lawin, and D. Löhlein.** Repeated administration of recombinant human insulin-like growth factor-I in patients after gastric surgery effect on metabolic and hormonal patterns. *Ann. Surg.* 222: 646–653, 1995.
12. **Gore, D. C., and F. Jahoor.** Glutamine kinetics in burn patients, comparison with hormonally induced volunteers. *Arch. Surg.* 129: 1318–1323, 1994.
13. **Griffiths, R. D.** Outcome of critically ill patients after supplementation with glutamine. *Nutrition* 13: 752–754, 1997.
14. **Hammarqvist, F., J. Wernerman, A. Rustom, A. von der Decken, and E. Vinnars.** Addition of glutamine to total parenteral nutrition after elective abdominal surgery spares free glutamine in muscle, counteracts the fall in muscle protein synthesis and improves nitrogen balance. *Ann. Surg.* 209: 455–461, 1989.
15. **Hankard, R. G., D. Darmaun, B. K. Sager, D. D'Amore, W. R. Parsons, and M. Haymond.** Response of glutamine metabolism to exogenous glutamine in humans. *Am. J. Physiol. Endocrinol. Metab.* 269: E663–E670, 1995.
16. **Hankard, R. G., M. W. Haymond, and D. Darmaun.** Effect of glutamine on leucine metabolism in humans. *Am. J. Physiol. Endocrinol. Metab.* 271: E748–E754, 1996.
18. **Jackson, N. C., P. V. Carroll, D. L. Russell-Jones, P. H. Sönksen, D. F. Treacher, and A. M. Umpleby.** The metabolic consequences of critical illness: acute effects on glutamine and protein metabolism. *Am. J. Physiol. Endocrinol. Metab.* 276: E163–E170, 1999.
19. **Jeevanandam, M., N. J. Holaday, and S. R. Petersen.** Integrated nutritional, hormonal, and metabolic effects of recombinant human growth hormone (rhGH) supplementation in trauma patients. *Nutrition* 12: 777–787, 1996.
20. **Jepson, M. M., P. C. Bates, P. Broadbent, J. M. Pell, and D. J. Millward.** Relationship between glutamine concentration and protein synthesis in rat skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* 255: E166–E172, 1988.
21. **Knaus, W. A., E. A. Draper, D. P. Wagner, and J. E. Zimmerman.** APACHE II: a severity of disease classification system. *Crit. Care Med.* 13: 818–829, 1985.
22. **Lacy, J. M., and D. W. Wilmore.** Is glutamine a conditionally essential amino acid? *Nutr. Rev.* 48: 297–309, 1990.
23. **Liberman, S. A., G. E. Butterfield, D. Harrison, and A. R. Hoffman.** Anabolic effects of recombinant insulin-like growth factor-I in cachetic patients with acquired immunodeficiency syndrome. *J. Clin. Endocrinol. Metab.* 78: 404–410, 1994.
24. **Long, C. L., K. M. Nelson, D. B. DiRenzo, J. K. Weis, R. D. Stahl, T. D. Broussard, W. L. Theus, J. A. Clark, T. W. Pinson, J. W. Geiger, H. L. Laws, W. S. Blakemore, and R. P. Carraway.** Glutamine supplementation of enteral nutrition: impact on whole body protein kinetics and glucose metabolism in critically ill patients. *J. Parent. Enteral Nutr.* 19: 470–476, 1995.
25. **MacBurney, M., L. S. Young, T. R. Zeigler, and D. W. Wilmore.** A cost evalutation of glutamine supplemented parenteral nutrition in adult bone marrow transplant patients. *J. Am. Diet. Assoc.* 94: 1263–1266, 1994.
26. **MacLennan, P. A., R. A. Brown, and M. J. Rennie.** A positive relationship between protein synthetic rate and intracellular glutamine concentration in perfused rat muscle. *FEBS Lett.* 215: 187–191, 1987.
27. **Matthews, D. E., H. P. Schwartz, R. D. Yang, K. J. Motil, V. R. Young, and D. M. Bier.** Relationship of plasma leucine and α-ketoisocaproate during a L-[1-^{13}C]leucine infusion in man: a method for measuring intracellular leucine tracer enrichment. *Metabolism* 31: 1105–1112, 1982.
28. **Mjaaland, M., K. Unneberg, J. Larsson, L. Nilsson, and A. Revhaug.** Growth hormone after abdominal surgery attenuated forearm glutamine, alanine, 3-methyl histidine, and total amino acid efflux in patients receiving total parenteral nutrition. *Ann. Surg.* 217: 413–422, 1993.
29. **Parry-Billings, M., J. Evans, P. C. Calderans, and E. A. Newsholme.** Does glutamine contribute to immunosuppression after major burns? *Lancet* 336: 523–525, 1990.
30. **Petersson, B., S. O. Waller, E. Vinnars, and J. Wernerman.** Long term effect of glycyl-glutamine after elective surgery on free amino acids in muscle. *J. Parent. Enteral Nutr.* 18: 320–325, 1994.
31. **Ross, R. J. M., and D. W. Wilmore.** Endocrinology in catabolic illness. *Endocrinol. Metab.* 3, *Suppl. A*: 115–118, 1996.
32. **Sönksen, P. H.** Double antibody technique for the simultaneous assay of insulin and growth hormone. In: *Hormones in Human Blood: Detection and Assay.* Cambridge, MA: Harvard University Press, 1976, p. 176–199.
33. **Stehle, P., J. Zander, N. Mertens, S. Albers, C. H. Puchstein, P. Lawn, and P. Fürst.** Effect of parenteral glutamine supplements on muscle glutamine loss and nitrogen balance after major surgery. *Lancet* 1: 221–233, 1989.
34. **Takala, J., E. Ruokonen, N. R. Webster, M. S. Nielsen, D. F. Zandstra, G. Vundelinckx, and C. J. Hinds.** Increased mortality associated with growth hormone treatment in critically ill adults. *N. Engl. J. Med.* 341: 785–791, 1999.
35. **Tremel, H., B. Kienle, L. S. Weilemann, P. Stehle, and P. Fürst.** Glutamine dipeptide supplemented parenteral nutrition maintains intestinal function in the critically ill. *Gastroenterology* 107: 1595–1601, 1994.
36. **Van der Hulst, R. R., B. K. van Kreel, M. F. von Meyenfeldt, R. J. M. Brummer, J.-W. Arends, N. E. P. Deutz, and P. B. Soeters.** Glutamine and the preservation of gut integrity. *Lancet* 334: 1363–1365, 1993.
37. **Vinnars, E., J. Bergström, and P. Fürst.** Influence of the post operative state on the intracellular free amino acids in human muscle tissue. *Ann. Surg.* 182: 665–671, 1975.
38. **Walser, H.** Misinterpretation of nitrogen balances when glutamine stores fall or are replenished. *Am. J. Clin. Nutr.* 53: 1337–1338, 1991.
39. **Wernerman, J., F. Hammarqvist, and E. Vinnars.** α-Ketoglutarate and postoperative muscle catabolism. *Lancet* 335: 701–703, 1990.
40. **Wolfe, R. R.** *Radioactive and Stable Isotope Tracers in Biomedicine: Principles and Practice of Kinetic Analysis.* New York: Wiley-Liss, 1992, p. 419–420.
41. **Zeigler, T. R., L. S. Young, K. Benfell, M. Scheltinga, K. Hortos, R. Bye, F. D. Morrow, D. O. Jacobs, R. J. Smith, J. H. Antin, and D. W. Wilmore.** Clinical and metabolic efficacy of glutamine supplemented parenteral nutrition after bone marrow transplantation. *Ann. Intern. Med.* 116: 821–828, 1992.